DALTON
COMMUNICATION

Inner- and outer-sphere binding of the minor groove site of adenine by alkali metal ions

Ashleigh E. Gibson,[a] Clayton Price,[a] William Clegg[b] and Andrew Houlton *[a]

[a] *Department of Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne, UK NE1 7RU. E-mail: andrew.houlton@ncl.ac.uk*

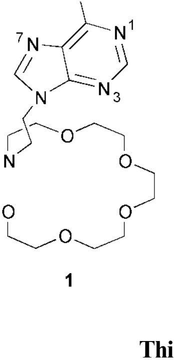
[b] *Crystallography Laboratory, Department of Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne, UK NE1 7RU. E-mail: w.clegg@ncl.ac.uk*

Received 17th September 2001, Accepted 6th December 2001
First published as an Advance Article on the web 17th December 2001

Na^+ and K^+ ions exhibit different modes of interaction with the N3 site of the DNA base adenine in a model system.

Alkali metal ions are ubiquitous in biology and, in particular, play an important role in nucleic acid chemistry.[1] To a large extent these metal ions are involved in charge neutralisation of the anionic backbone of (deoxy)ribonucleotide polymers. This enables the association of multiple strands as, for example, in duplex formation. However, more specific effects have been suggested, in particular with regards to the minor groove of B-DNA structures. Here the spine of hydration identified in the Dickerson–Drew duplex dodecamer[2] $d(CGCGAATTCGCG)_2$ has been reinterpreted as containing metal ions. The presence of monovalent cations in the minor groove in the AT sequence was observed theoretically,[3] by NMR,[4] and in X-ray studies by Williams[5] and Egli.[6] The ApA, TpA and ApT steps possess the most negative electrostatic potential of the various combinations of base-steps. As a result it has been suggested that these pockets can bind otherwise mobile cations.[3] In light of this, and subsequent investigations, it has been proposed that alkali metal ion···DNA minor groove interactions may be a source of sequence-dependent effects, such as the narrowing of the groove at A-tracts.[3]

We have been using a range of models for probing the interaction of d-block metal ions with purine sites located in the minor groove of DNA.[7] These compounds contain an additional metal ion-binding site tethered at the position of the deoxyribose ring. This restricts intramolecular co-ordination to sites adjacent to N9. Given this interest in the interactions of alkali metal ions with the A–T pair we have attached the 18-aza-crown to 9-ethyladenine, **1**, in an effort to examine the purine component of this base pair. Azacrown macrocycles of this type are known to bind Group I ions and have been used extensively by Gokel[8] to probe ion···biomolecule interactions. Here we report the preparation and molecular structures of Na^+ and K^+ complexes of **1**.† Interestingly, a different mode of interaction between the metal ions and the nucleobase is seen in each case. Moreover, DFT calculations show how these have quite different effects on the electronic structure of the nucleobase.



1

Ligand **1** was prepared by alkylation of 18-aza-crown using chloroethyladenine. The sodium salt, **1a**, was obtained by addition of $NaBPh_4$ to **1** in MeCN.† X-Ray quality crystals were obtained by slow cooling of **1a** in an EtOH–H_2O mixture (95 : 5).‡ The potassium complex, **1b**, was prepared similarly, with KPF_6 in place of $NaBPh_4$.

The molecular structure of **1a** reveals a seven-co-ordinate Na^+ ion (Fig. 1) with the aza-crown contributing six of the donor atoms and a water molecule occupying the remaining site. The Na–O_{Crown} bond lengths range from 2.544 to 2.724 Å (mean 2.608 Å) and the single Na–N distance is 2.930 Å. The shortest metal–ligand bond length is Na–OH_2 at 2.327 Å. The co-ordinated water molecule forms a hydrogen bond to the N3 site of the adeninyl moiety with an $H_2O \cdots N3$ separation of 2.836 Å and an ∠N3–H–O of 175.6°.

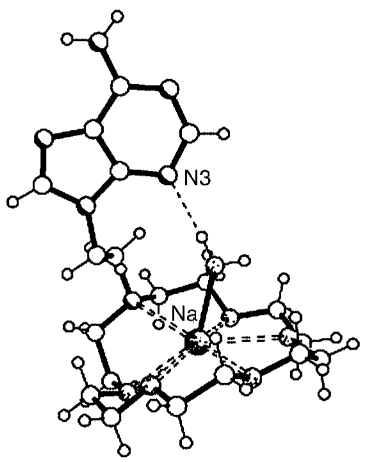


Fig. 1 Molecular structure of the cation **1a** highlighting the second-sphere interaction with N3.

In marked contrast the potassium complex, **1b**, contains a direct metal–adenine interaction (Fig. 2). The metal ion is again co-ordinated by the azacrown with K^+–O distances ranging from 2.622 to 2.816 Å (mean 2.755 Å) and the K^+–N distance of 3.149 Å. There are also close contacts with the PF_6^- anion at 2.811 and 3.033 Å. In addition, the N3-site of adenine co-ordinates the metal ion with a distance of 2.939 Å. However, the nature of the interaction appears to be unusual in that the metal ion is significantly out of the plane of the nucleobase (∠ K–N3–C6 124.1°) suggesting a highly ionic character to the bonding.§

To gain an insight into the electron redistribution induced by these different modes of interaction density functional calculations¶ (DFT) were performed on 9-methyladenine, **1a**, **1b** and N3-protonated 9-MeA. Perhaps of most interest are the changes to the electrostatic-potential (ESP) atomic charges at the nucleobase hydrogen bonding sites (Table 1). In all cases, the ESP charges on the exocyclic amino protons becomes more

DOI: 10.1039/b108427p

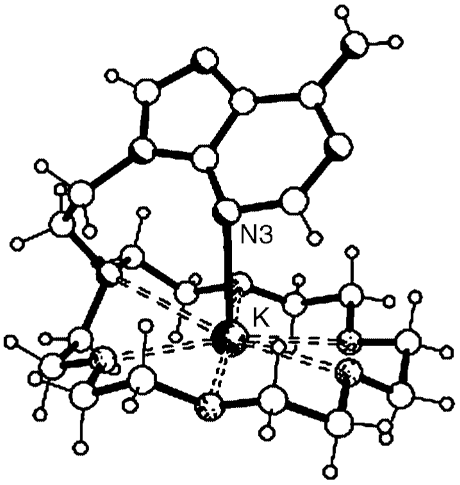


Fig. 2 Molecular structure of the cation **1b** highlighting the metal ion–N3 interaction.

positive compared to 9-MeA. However, the magnitude of the effect is dependent on the type of interaction and decreases in the following order: H^+–N3 > M–N3 > M–$OH_2 \cdots$ N3 (ΔESP averaged over both protons +0.045; +0.038; +0.034 e, respectively). Both inner- and outer-sphere binding affect the exocyclic amino group structurally, increasing the resonance with the aromatic ring resulting in a more planar geometry (sum of angles at the exocyclic NH_2 group: $9MeA_{Calc}$ 353.2°; $\mathbf{1a}_{X\text{-ray}}$ 360.0; $\mathbf{1a}_{Calc}$ 359.9°; $\mathbf{1b}_{X\text{-ray}}$ 359.37°; $\mathbf{1b}_{Calc}$ 359.2°). This effect is also seen in a shortening of the N6–C6 bond length. The effect of these perturbations of the adeninyl moiety can be seen in Fig. 3, which shows the calculated electrostatic potential mapped onto the molecular surface for each of 9MeA, **1a** and **1b**, respectively. Most evident is the loss of lone pair character at the N6-amino group. The mode of ion interaction also brings about small but different effects on the ESP atomic charges on N1 and N7.

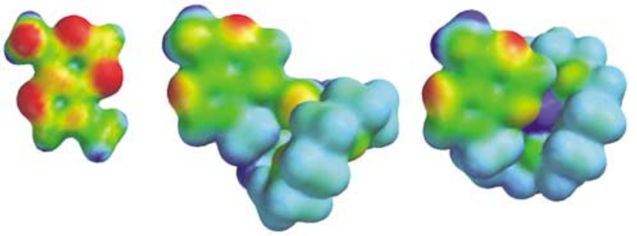


Fig. 3 Electrostatic potentials mapped on to the electron density isosurfaces of 9-MeA, **1a** and **1b** calculated at the BP/LACVP* level of theory. The potential energy range is 10 hartrees (red) to 100 hartrees (blue) in each case. The electron density isovalue was varied to facilitate comparison; 9-MeA 0.02 e au^{-3}, while for **1a** and **1b** it was set lower (0.005 e au^{-3}) due to the cationic charge of these molecules. Note the effect on the potential due to the lone pair at the exocylic amino group.

In conclusion, these results highlight an interesting difference between Na^+ and K^+ ions in their interaction with N3 of adenine in this model system. We do not however presume to rule out the reverse cases, *e.g.* K–$OH_2 \cdots$ N3A, and also realize that the situation for the AT base pair is rather different where the more electronegative O2-T is also available. However, both inner- and outer-sphere interactions appear to be important as shown in recent MD-simulations with both types of interactions being involved in narrowing of the groove.[11] An explanation for the difference seen in **1a** and **1b** is not obvious. It would be simple to suggest this is due to some forced interaction imposed by ligand system in the case of K^+. However, models show that the increase in ionic radius (Na^+ = 1.95 *versus* K^+ = 2.66 Å) does not prohibit the formation of the K^+ analogue of **1a** and the comparison with other data[§] suggests that the out-of-plane binding is not an artifact. Further work is required to understand these obviously subtle effects.

Table 1 Electrostatic-potential fitted atomic charges calculated at the BP-LACVP* level of theory and selected metrical parameters. Data in parentheses are from the X-ray crystal structure data (Å)

Atom	9-MeA	1a	1b	9-MeA–N3H$^+$
N1	−0.634	−0.570	−0.616	−0.497
N3	−0.625	−0.470	−0.471	−0.421
N7	−0.458	−0.454	−0.497	−0.417
N6	−0.760	−0.780	−0.807	−0.702
H6a	0.364	0.396	0.406	0.412
H6b	0.360	0.393	0.394	0.402
M–N3		4.68 (4.792)	2.81 (2.939)	
O–N3		2.80 (2.836)		
N6–C6	1.37	1.35 (1.347)	1.35 (1.335)	1.34
$\Sigma\angle$HNH/°	353.2	359.9	359.2	360.0

Notes and references

[†] **1**: N^9-(2-Chloroethyl)adenine (0.36 g; 1.8 mmol) and 1-aza-18-crown-6 (0.13 g; 0.49 mmol) were suspended in propanonitrile (10 ml). Sodium carbonate (0.29 g; 2.75 mmol) and sodium iodide (15 mg; 0.1 mmol) were added and the reaction was heated to reflux for 18 h. After cooling to rt the solids were filtered and washed with MeCN (25 ml). The filtrate was evaporated and the residue columned on silica eluting with 5 to 20% MeOH–CH_2Cl_2. The product, obtained as a white solid in 69.5% yield, was recrystallised from EtOAc–MeCN. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.3 (s, 1H, H_2), 7.95 (s, 1H, H_8), 5.95 (br s, 2H, NH_2), 4.3 (t, 2H, J = 5.9 Hz, N^9CH_2), 3.8–3.5 (m, 20H), 2.95 (t, 2H, J = 5.9 Hz, NCH_2), 2.6 (t, 4H, J = 4.8 Hz, NCH_2). MS m/z 425.5 [M + H], 447.5 [M + Na].

1a: Sodium salt, **1a**, was obtained by addition of $NaBPh_4$ to **1** in MeCN and isolated by concentration and precipitation with ether. X-Ray quality crystals of **1a** were obtained by slow cooling of an EtOH–H_2O mixture (95 : 5): Elem. anal.: Found C, 66.02; H, 6.95; N, 10.65. Calc. for $C_{43}H_{54}BN_6NaO_6{\cdot}H_2O$ C, 65.79; H, 6.94; N, 10.72%. MS m/z 447.2 [**1**Na]$^+$

1b: The potassium complex, **1b**, was prepared similarly, though KPF_6 replaced the $NaBPh_4$. X-Ray quality crystals of **1b** were again obtained by slow cooling of an EtOH–H_2O mixture (95 : 5). Elem. anal.: Found C, 37.31; H, 5.18; N, 13.59. Calc. for $C_{19}H_{32}N_6O_5KPF_6$ C, 37.48; H, 5.30; N, 13.81%. MS m/z 463 [**1b** − PF_6]$^+$, 609 [**1b** + H]$^+$, 647 [**1b** + K]$^+$.

[‡] Crystal data for **1a**: $C_{43}H_{54}BN_6NaO_6$, M = 784.7, monoclinic, space group $P2_1/n$, a = 15.1053(13), b = 13.3332(12), c = 22.2103(19) Å, β = 108.227(2)°, U = 4248.8(6) Å^3, Z = 4, D_c = 1.227 g cm^{-3}, μ = 0.091 mm^{-1} (Mo-Kα, λ = 0.71073 Å), T = 160 K; $R(F, F^2 > 2\sigma)$ = 0.039, $R_w(F^2$, all data) = 0.110 for all 9827 unique data (27020 measured, R_{int} = 0.021, $\theta < 28.4°$, CCD diffractometer) and 527 refined parameters.

Crystal data for **1b**: $C_{19}H_{32}F_6KN_6O_5P$, M = 608.6, monoclinic, space group $P2_1/c$, a = 8.5521(7), b = 18.2322(16), c = 17.0228(15) Å, β = 99.177(2)°, U = 2620.3(4) Å^3, Z = 4, D_c = 1.543 g cm^{-3}, μ = 0.349 mm^{-1} (Mo-Kα, λ = 0.71073 Å), T = 160 K; $R(F, F^2 > 2\sigma)$ = 0.045, $R_w(F^2$, all data) = 0.106 for all 6119 unique data (22262 measured, R_{int} = 0.030, $\theta < 28.5°$, CCD diffractometer) and 349 refined parameters. Programs: standard Bruker AXS control and integration software and SHELXTL.[9] CCDC reference numbers 167590 and 167591. See http://www.rsc.org/suppdata/dt/b1/b108427p/ for crystallographic data in CIF or other electronic format.

[§] The apparently unusual out-of-plane interaction appears less so after a survey of the Cambridge Crystallographic Database. Two types of M_{group1}–$N3_{adenine}$ interactions are found, the hydrated potassium and rubidium salts of adenosine-5-diphosphate (K^+ salt, Refcode = KADPHD01;[10a] Refcode = KADPHD02[10b,c]). The K–N3 separation and ∠ K–N3–C6 are KADPHD01 3.151 Å, 149.11°, KADPHD02 3.162 Å, 150.65°. In these cases there are no obvious constraints to the metal–ligand geometry but still out-of-plane binding is found. This suggests that the metal–nucleobase interaction in **1b** is not simply an artifact imposed by the aza-crown ligand.

[¶] Density functional calculations (DFT) were performed using the Titan program package (Wavefunction Inc., USA, 1999, version 1.0.5). Geometries were optimised at the Becke–Perdew level of theory using the LA-CVP* basis set. Additional calculations on **1b** whereby the K^+ ion was located more in the plane of the adeninyl group converged (K–N3–C6 168.1°; K–N3 2.960 Å. ESP atomic charges: N1 −0.593; N3 −0.491; N7 −0.491; N6 −0.753; H6a 0.392; H6b 0.382; K 0.503). However, this structure was 2.8 kJ mol^{-1} higher in energy than that reported in Table 1.

1 G. M. Blackburn and M. J. Gait eds., *Nucleic Acids in Chemistry and Biology*, IRL Press, Oxford, 1990.

2 H. R. Drew and R. E. Dickerson, *J. Mol. Biol.*, 1981, **151**, 535.

3 M. A. Young, B. Jayaram and D. L. Beveridge, *J. Am. Chem. Soc.*, 1997, **119**, 59; D. Hamelberg, L. McFail-Isom, L. D. Williams and W. D. Wilson, *J. Am. Chem. Soc.*, 2000, **122**, 10513.

4 N. V. Hud, V. Sklenar and J. Feigon, *J. Mol. Biol.*, 1999, **286**, 651; V. P. Denisov and B. Halle, *Proc. Natl. Acad. Sci.*, 2000, **97**, 629.

5 L. McFail-Isom and L. D. Williams, *Curr. Opin. Struct. Biol.*, 1999, **9**, 298; K. Woods, L. McFail-Isom, C. C. Sines, S. B. Howerton, R. K. Stephens and L. D. Williams, *J. Am. Chem. Soc.*, 2000, **122**, 1546.

6 V. Tereshko, G. Minasov and M. Egli, *J. Am. Chem. Soc.*, 1999, **121**, 3590; V. Tereshko, C. J. Wilds, G. Minasov, T. P. Prakash, M. A. Maier, A. Howard, Z. Wawrzak, M. Manoharan and M. Egli, *Nucleic Acids Res.*, 2001, **29**, 1208.

7 (*a*) C. Price, M. R. J. Elsegood, W. Clegg and A. Houlton, *J. Chem. Soc., Chem. Commun.*, 1995, 2285; (*b*) C. Price, M. R. J. Elsegood, W. Clegg, N. H. Rees and A. Houlton, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1762; (*c*) M. A. Shipman, C. Price, A. E. Gibson, M. R. J. Elsegood, W. Clegg and A. Houlton, *Chem. Eur. J.*, 2000, **6**, 4371; (*d*) M. A. Shipman, C. Price, M. R. J. Elsegood, W. Clegg and A. Houlton, *Angew. Chem., Int. Ed.*, 2000, **39**, 2360; (*e*) C. Price, C. M. A. Shipman, S. L. Gummerson, A. Houlton, W. Clegg and M. R. J. Elsegood, *J. Chem. Soc., Dalton Trans.*, 2001, 353; (*f*) C. Price, M. A. Shipman, N. H. Rees, M. R. J. Elsegood, A. J. Edwards, W. Clegg and A. Houlton, *Chem. Eur. J.*, 2001, **7**, 1194.

8 O. F. Schall and G. W. Gokel, *J. Am. Chem. Soc.*, 1994, **116**, 6089; O. F. Schall and G. W. Gokel, *J. Org. Chem.*, 1996, **61**, 1449; S. L. De Wall, L. J. Barbour, O. F. Schall and G. W. Gokel, *J. Chem. Crystallogr.*, 2000, **30**, 227; E. S. Meadows, S. L. De Wall, L. J. Barbour and G. W. Gokel, *J. Am. Chem. Soc.*, 2001, **123**, 3092.

9 G. M. Sheldrick, SHELXTL, version 5.1, Siemens Analytical Instruments, Madison, WI, 1998.

10 (*a*) D. A. Adamiak and W. Saenger, *Acta Crystallogr., Sect. B*, 1980, **36**, 2585; (*b*) P. Swaminathan and M. Sundaralingam, *Acta Crystallogr., Sect. B*, 1980, **36**, 2590; (*c*) M. A. Viswamitra, M. V. Hosar and O. Kennard, *Cryst. Struct. Commun.*, 1976, **5**, 819.

11 D. Hamelberg, L. McFail-Isom, L. D. Williams and W. D. Wilson, *J. Am. Chem. Soc.*, 2000, **122**, 10513.