DNA structure: cations in charge?

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Recent X-ray diffraction, NMR spectroscopy and molecular mechanics results suggest that monovalent cations selectively partition into the minor groove of AT-tracts in DNA. These observations are consistent with DNA deformation by electrostatic collapse around areas of uneven cation density. This model predicts the occurrence of known DNA deformations, such as AT-tract bending and changes in the minor-groove width.

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Abbreviation
NDB Nucleic Acid Database

Introduction

Few would argue against the importance of electrostatic interactions in DNA bending, twisting, groove-width variation, deformation and condensation, and in RNA folding and catalysis. A central role for electrostatics in bending mechanisms is indicated by the effects of salt on the bending of AT-tracts [1–3] and GC-tracts [4–6]. Yet, the analysis of high-resolution DNA structures, originating with 'Calladine's rules' [7], has focused primarily on direct base–base interactions, implicitly discounting contributions from electrostatics. The limitations on electrostatic analysis are partially technical; one simply cannot obtain much information about shielding, counterion positions or electrostatic forces. These limitations provide a partial explanation for the durability of the base–base paradigm [8•], in spite of its obvious deficiencies. Some current difficulties in the treatment of electrostatic interactions in nucleic acids have been discussed by Feig and Pettitt [9]. Here, we review recent proposals of unanticipated roles for cations in the control and perturbation of B-DNA structures. Space limitations prohibit the review of exciting new developments in the realm of the cation control of quadruplex structure (for example, see [10]).

Lost cations

A full complement of neutralizing cations is contained within every nucleic acid crystal; net charge would explode a crystal. Yet, historically, the cations that neutralize the anionic phosphates of nucleic acids, especially alkali monovalent cations, have been omitted from three-dimensional structures. The seminal 'Dickerson dodecamer' (CGCGAATTCGCG, 2.5 Å resolution, Nucleic Acid Database [NDB] entry DBL001 [11]), with 22 anionic groups, lacks any cationic counterions. Even at very high resolution (1.4 Å), Williams and co-workers [12••,13••] could directly observe only one magnesium ion and a partial spermine molecule among over 150 water molecules associated with the Dickerson dodecamer (NDB entry BDL084). Similarly, the 76 phosphates of the highest resolution tRNA structure (2.5 Å, NDB entry trna10 [14,15]) are predominantly un-neutralized. The tRNA structure contains only four magnesium ions and no monovalent cations. This apparent charge imbalance persists even in complexes of nucleic acids with cationic proteins. In the nucleosome core particle (2.8 Å, NDB entry pd0001 [16]), 290 phosphate groups are compensated by only 162 cationic amino acids and six divalent cations. This count underestimates the imbalance by ignoring anionic amino acid residues.

Found cations

Where are the monovalent cations that surround DNA and RNA? Williams and co-workers [12••,13••] have proposed a hybrid-solvent model that is consistent with the near invisibility of monovalent cations to X-ray diffraction. In this model, solvent sites that were previously characterized as pure water are, in fact, hybrids and are partially occupied by monovalent cations. A hybrid is composed of several kinds of atoms that occupy symmetry-equivalent sites. Solvent sites surrounding DNA are occupied by water molecules at some locations in the crystal, but are occupied by monovalent cations at locations that are equivalent by symmetry. The cation occupancy of a hybrid-solvent site is essentially equivalent to the local stoichiometric ratio of monovalent cations to water molecules. That ratio averages less than 10% for solvent sites in DNA crystals.

We obtain this estimate of the stoichiometric ratio of water to monovalent cations using two independent back-of-the-envelope calculations. First, assume that the unit cell of a dodecamer crystal contains one monovalent cation for every phosphate (1.5×10^{-22} mol phosphate per unit cell) and that half the volume of the unit cell ($V/2 = 3.3 \times 10^{-23}$ L) is excluded by DNA and the other half contains saline solution. If so, the monovalent cation concentration is 4 M in 55 M water. This stoichiometric ratio suggests, if the cations are evenly distributed, 7% fractional occupancy of hybrid-solvent sites by monovalent cations. Crystals contain other cations, such as magnesium and spermine, however, which compete with monovalent cations for entry into the crystal. Therefore, 7% is the maximum average monovalent cation occupancy. Second, polymeric DNA in solution may not provide an ideal model for cation distribution around oligonucleotides, especially in crystals. Using polymeric DNA in solution as a guide, however, the concentration of monovalent cations adjacent to DNA is estimated to be 1 M, while water is 55 M, suggesting 2% fractional occupancy.

The overwhelming occupancies of water over monovalent cations in hybrid-solvent sites present difficult analytical challenges during X-ray structure determination. The X-ray scattering characteristics (electron density) of a hybrid are weighted by the fractional occupancies. If the coordinating ligands are pliable, the coordination geometry will also show hybrid characteristics, possibly manifested by high thermal factors. In fact, the relatively high thermal factors of DNA phosphate groups at low temperature [17] are entirely consistent with the hybrid-solvent model. Even in the absence of hybrid smearing, locating monovalent cations from geometric considerations is challenging because water molecules and monovalent cations, unlike divalent cations, both have pliable and unpredictable coordination geometries. The identification of sodium is most problematic because sodium ions and water molecules carry the same number of electrons, giving electron density peaks with similar volumes.

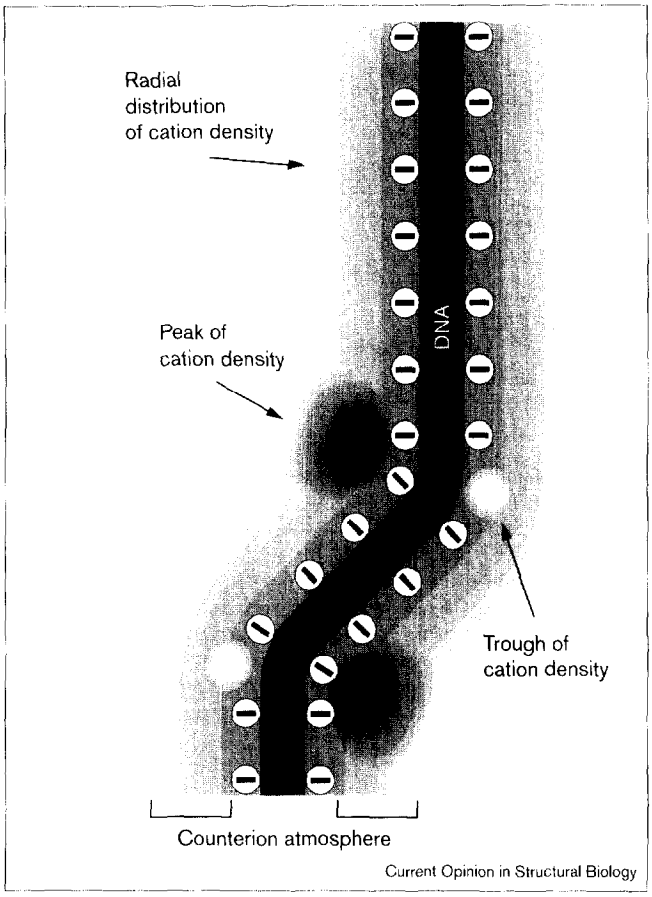
Structural versus solution studies

The convergence of structural and solution models would add predictive power to both approaches. Polyelectrolyte solution models, in which monovalent and divalent cations are distributed within delocalized 'atmospheres' [18], are consistent with much DNA physical chemistry. Structural models, in contrast, naturally focus on more ordered components, in part because delocalized systems are refractory to structural analysis.

In purely polyelectrolyte solution models, cation distributions around DNA are described by sequence-independent radial functions. Concentration falls off with increasing distance from the helical axis (see the top of Figure 1). Cations remain fully hydrated and do not bind with long lifetimes at specific sites [19,20••]. The structure-based hybrid-solvent model similarly suggests a distribution of monovalent cation density and a predominance of full hydration. The cations are largely delocalized and exhibit irregularity, suggesting nonsite binding. Yet, the strict aversion of fully hydrated magnesium ions for DNA amino groups [13••] indicates that a radial cation atmosphere of mobile, fully hydrated cations, whether monovalent or divalent, would be perturbed by sequence. Additional deviations from purely polyelectrolyte models are suggested by the partial dehydration and site binding of a fraction of the monovalent cations, especially within the minor groove of AT-tracts (see below). An unanticipated decoupling of dehydration and site binding is suggested by the presence of a restrictive geometric monolayer of solvent sites within the minor groove of AT-tracts [13••]. Monovalent cations would partially dehydrate upon entry into the minor groove, but would retain two-dimensional mobility.

The influences of the functional groups of DNA bases on cation distributions imply that systems of peaks and troughs would be superimposed on the radial function describing cation density in solution. The cation peaks and troughs would effectively be static, even though the population that comprises them is highly mobile, because the motions of water molecules [21] and cations [22] are rapid on the timescale of DNA motions [23–25].

Figure 1

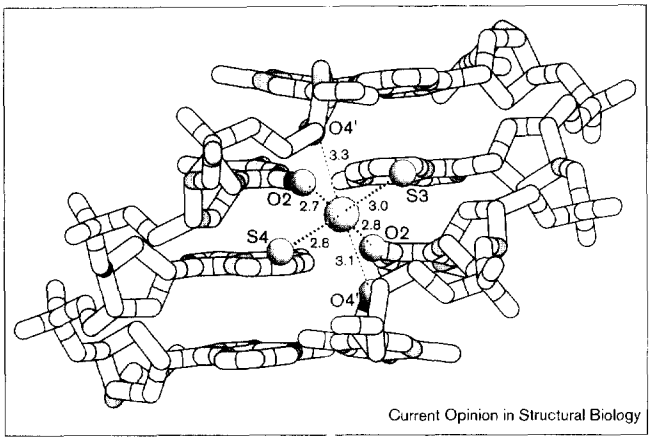


A system of sequence-induced peaks and troughs of cation density superimposed on the radial polyelectrolyte distribution surrounding DNA. DNA bends around peaks of cation density and bends away from troughs. The concentration of cationic charge in the counterion atmosphere is indicated by a shade of gray, with black indicating regions of greatest concentration.

Monovalent cations in the minor groove of AT-tracts

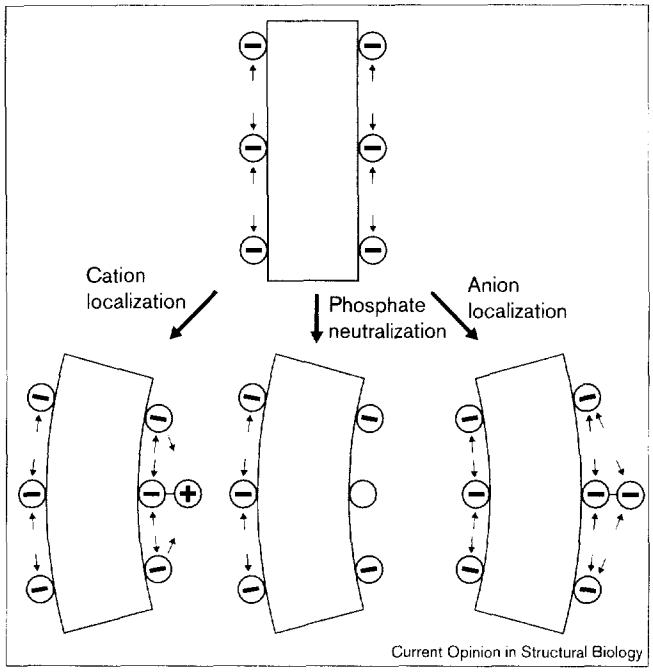
Williams and co-workers [12••,13••] have recently attempted to use X-ray diffraction both to determine cation occupancies and to search for peaks and troughs of cation density around DNA. The minor groove of AT-tract DNA was a reasonable place to anticipate peaks of monovalent cation density. Many years ago, Rich and co-workers [26] used single-crystal diffraction to identify a sodium ion positioned near the floor of the abbreviated minor groove of a dinucleotide duplex. The relevance of that structure was discounted during initial interpretations of the Dickerson dodecamer. Those interpretations described a purely aqueous 'spine of hydration' in the AT-tract minor groove [27]; however, Bartenev *et al.* [28] inferred that cesium localizes in AT-tract minor grooves using fiber diffraction of polymeric DNA. Recently determined high-resolution single crystal diffraction data for several salts

Figure 2



View into the minor groove of the potassium form of $[dCGCGAATTCGCG]_2$, showing the coordination geometry of the 5′ ApT 3′ step. DNA atoms are shaded by type, with nitrogen (dark) > oxygen > carbon > phosphorous (light). The ligands of the water–cation hybrid, which are represented as spheres, are two O4′ atoms, two carbonyl oxygen atoms (O2) and two occupants of the secondary hydration layer (S). The sphere representing the water–cation hybrid is larger and darker than the other six spheres. Distances indicated are in angstroms. Adapted from [13••].

Figure 3



Schematic representation of electrostatic collapse, showing equivalent effects of cation localization, phosphate neutralization and anion localization. The schematic is intended to indicate the covalent attachment of cations or anions to DNA. Only force vectors between nearest neighbors are indicated.

of the Dickerson dodecamer led to a re-interpretation, suggesting significant occupancy by either sodium [12••] or potassium [13••] in the minor groove. Work by the same group using rubidium and cesium has confirmed monovalent occupancy within the minor groove (LD Williams *et al.*, unpublished data). The fiber and crystal data are supported by results obtained on DNA in solution. Hud *et al.* [29••] demonstrated that ammonium binds preferentially in AT-tract minor grooves. These authors established isotopically labeled ammonium as an excellent NMR probe for monovalent alkali ions in both B-DNA and quadruplex DNA [30]. The combined experimental results are consistent with a series of nanosecond-level molecular dynamics simulations, carried out by Young and Beveridge [31••,32••], of DNA fragments under various salt conditions. In those molecular dynamics simulations, monovalent cations bind preferentially in AT-tract minor grooves.

In summary, 1998 was a water-shed year, during which it became clear that the 'spine of hydration' in AT-tracts can have significant monovalent cation occupancy. The six-coordinate site at 5′ ApT 3′ steps forms an especially good location for hard monovalent cations, with four oxygen atoms from DNA and two oxygen atoms from water molecules as ligands (Figure 2). The ligands localize the cation in a region that is recognized as having unusually high electronegative potential [31••,32••,33,34]. The coordination distances at the ApT step are slightly greater than expected for a potassium ion, but are greater in number and, in some cases, are shorter than would be expected for a water molecule. Indeed, the ApT binding site meets the monovalent binding criteria of Draper and Misra [35••].

Cation density

Peaks imply troughs. The observation of high monovalent cation density in the minor groove of AT-tracts suggests that cation density is depleted elsewhere. The positions and amplitudes of peaks and troughs of monovalent cation occupancy would depend on numerous factors: DNA coordination geometries and electronegative potentials, as modulated by sequence and conformation; cation size and dehydration energetics; and the concentrations of the competing multivalent cations, polyamines, minor-groove binders, proteins and so on.

Electrostatic collapse

It is reasonable to propose a model in which nucleic acids are deformed by uneven cation density (see the bottom of Figure 1) within the counterion atmosphere. The deformation can be global, as in axial bending, and local, as in groove-width variation. One mechanism, originally proposed by Mirzabekov, Rich and co-workers [36,37] to explain spontaneous DNA bending around proteins, is known as 'electrostatic collapse'. The model is supported by the elegant experiments of Maher and co-workers [6,38–40], who demonstrated that the electronic effects of protein binding can be isolated from other effects by the construction of 'phantom proteins'. Maher and colleagues [6,38–40] have shown that covalently localized cations and neutralized phosphates can bend DNA in the absence of proteins.

The electrostatic collapse phenomenon has been subdivided into various classes, although a unifying view is obtained by considering the summation of local coulombic force vectors. A schematic diagram showing how axial bends result from phosphate neutralization, cation localization or anion localization is shown in Figure 3. Electrostatic collapse is consistent with polyelectrolyte models. Polyelectrolyte models predict that one can induce a net uptake or release of cationic counterions by altering the anionic charge density of DNA [18]. The electrostatic collapse model states the converse; that one can locally change the anionic charge density (i.e. bend the DNA) by externally modulating the local concentration of cationic counterions.

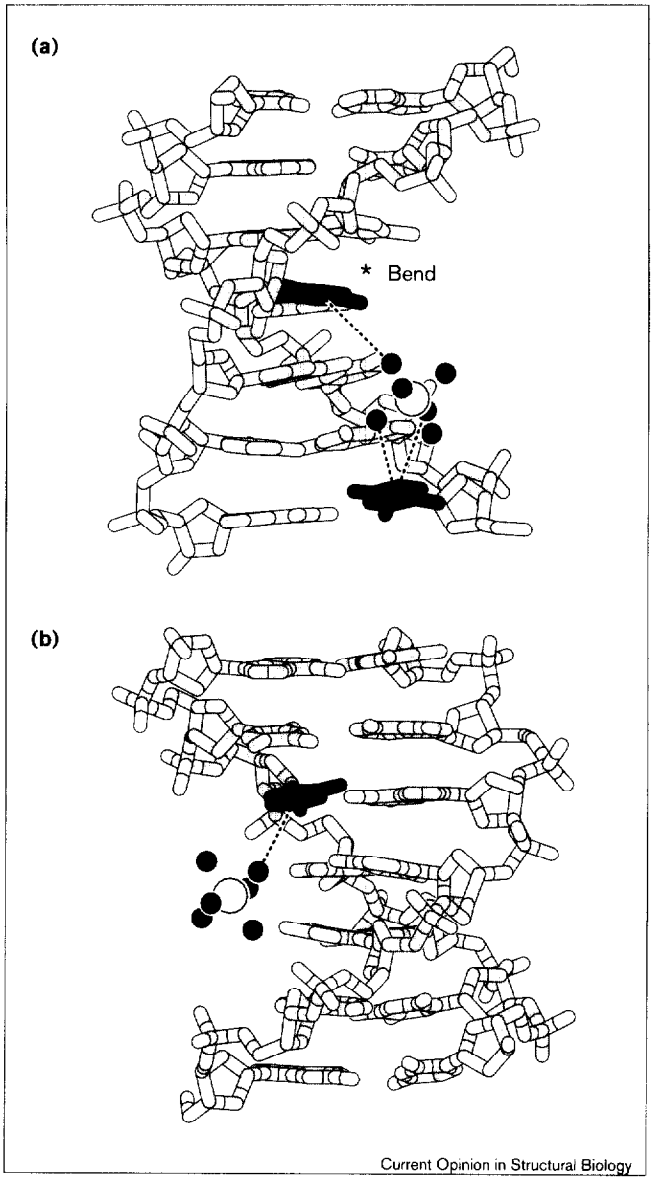
Experimentally, the degree and direction of DNA bending can be controlled by systematically varying the location and amplitude, from positive to negative, of the peaks of cationic density around DNA. This variation was accomplished by Strauss-Soukup and Maher [41••] using appropriate substitutions of cationic or anionic residues in the transcription factor GCN4 bound to DNA. Gold and co-workers [42,43] are developing methodologies to control the site of the localization of covalently attached charge on DNA. In the final analysis, one must account for the distributions of mobile counterions in order to achieve a quantitative and fully reliable model of DNA bending by 'phantom proteins'.

The issue of DNA deformation around mobile cations cannot be fully resolved by experiments in which the locations of charges are fixed by either protein or covalent attachment. An explicit treatment of the dynamical characteristics of divalent cations is described by Rouzina and Bloomfield [20••]. They propose that DNA is bent by short-range electrostatic interactions between phosphate groups and mobile divalent cations. An analogous conclusion was obtained by Stigter [34], who used a fixed dielectric and concluded that electrostatic forces act over a relatively long range -- up to two helical turns. The locations of the peaks of monovalent cation density within the minor groove of AT-tract DNA predict that the bending direction is towards the minor groove. A-tract DNA does indeed bend towards the minor groove in solution [44]. The locations of the peaks of monovalent cation density predict that AT-tract DNA will have a narrow minor groove. A-tract DNA does indeed have a narrow minor groove [45,46]. We believe that essentially unequivocal evidence establishing the importance of cation density in determining groove width is provided by Feigon and co-workers [29••] with their ammonium probe. They demonstrate that where the time-averaged density of monovalent cations in the minor groove is greatest, the groove is narrowest.

Competition between monovalent and divalent cations

Divalent cations compete with monovalent cations in solution [47–49], but do not necessarily bind preferentially at the same sites [29••,50••]. Differences in monovalent and divalent binding site preferences can be inferred by comparing dodecamer structures in crystals grown from solutions of relatively low [12••,13••] and high [51•] concentrations of magnesium. When the occupancies of divalent cations around DNA increase, the occupancies of monovalent cations decrease. Divalent cations outside the minor groove displace monovalent cations from within the minor groove.

Figure 4



Cation–π interactions in the major groove of distorted B-DNA. Dashed lines indicate cation–π interactions between DNA bases and $Mg(OH_2)_6^{2+}$. The DNA is in stick representation. The bases that engage in cation–π interactions are cytosines (blue). $Mg(OH_2)_6^{2+}$ is represented by spheres, with the radius of Mg^{2+} (yellow) greater than that of first shell water oxygens (red). **(a)** A portion of DNA dodecamer duplex d(CGCGAATTCGCG). **(b)** A portion of decamer duplex d(CGATCGATCG). The asterisk indicates the location of a bend in the helical axis. Adapted from [57••].

Surprising roles for monovalent cations

The observation of specific interactions between DNA and monovalent cations has broad implications. Specific cation–DNA interactions appear to mediate recognition processes. Ladbury and co-workers [52••] observed that monovalent cations are recruited in order to stabilize a specific protein–DNA complex. They propose that cations mediate specific interactions between the TATA-box-binding protein and its DNA recognition sequence in a hyperthermophilic organism that lives at high salt concentrations. Doudna and co-workers [53••] observed a specific binding site for monovalent metal ions within a catalytic RNA. Similarly, Chaires and co-workers have calorimetrically detected modest, but real effects of monovalent ion identity on the affinity of daunomycin for DNA (J Chaires *et al.*, personal communication). These calorimetric data are consistent with the observations of Wang *et al.* [54], who reported a sodium chelation site formed by daunomycin and an adjacent guanine (reviewed in [55]).

Roles for divalent cations in condensation

Clark *et al.* (GR Clark, CJ Squire, RF Martin, J White, personal communication) have developed a powerful crystalline model for DNA condensation *in vivo*. They performed a controlled and stepwise dehydration of DNA dodecamer crystals and observed increasing crystalline order and compaction. The most dehydrated crystals exist as infinite polymeric networks, in which adjacent duplexes are cross-linked by coordinate bonds through partially dehydrated magnesium ions. The importance of specific cation interactions in DNA condensation is reinforced by the observation of Subirana and co-workers [56] that changing from magnesium to calcium counterions switches the packing mode in dodecamer crystals.

Roles for divalent cations in base unstacking

Williams and co-workers [57••] proposed that inorganic cations engage in cation–π interactions with the bases of DNA and RNA. For example, hydrated magnesium ions located in the major groove of B-DNA appear to partially pull out cytosine bases from the helical stack, exposing π systems to positive charge (Figure 4). This proposed unstacking function for inorganic cations is a rediscovery of a phenomenon reported over 30 years ago by Mildvan and co-workers [58], who found evidence that $Mn(H_2O)_6^{2+}$ intercalates in ApU, separating the bases by over 7.0 Å. Williams and co-workers [57••] suggest that cation–π interactions contribute to the stability of a wide range of RNA structures, including the anticodon arm of yeast $tRNA^{Phe}$ and the *Tetrahymena* group I intron. This unstacking function opposes generally accepted roles for cations in stabilizing DNA and RNA duplexes and higher assemblies, and suggests a number of new mechanistic roles for cations in DNA bending, DNA–protein recognition, base flipping and RNA folding and catalysis.

Conclusions

The influences of cations on nucleic acid structure are multifaceted and subtle. Cations can simultaneously stabilize and unstack. Cations can cause static bends from dynamical states. With the increased availability of high flux X-ray sources and sensitive detectors, and a newfound focus among the structural community, we anticipate the day when all the cations will be found.

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