

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231528463>

# Stereochemistry of Binding of Metal Cations and Water to a Phosphate Group

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · JANUARY 1998

Impact Factor: 12.11 · DOI: 10.1021/ja972237+

---

CITATIONS

29

---

READS

10

## 2 AUTHORS:



**Bohdan Schneider**

Institute of Biotechnology AS CR, Prague, Cz...

82 PUBLICATIONS 3,387 CITATIONS

SEE PROFILE



**Martin Kabelác**

University of South Bohemia in České Buděj...

49 PUBLICATIONS 1,733 CITATIONS

SEE PROFILE

# Stereochemistry of Binding of Metal Cations and Water to a Phosphate Group

Bohdan Schneider\* and Martin Kabeláč

Contribution from the J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, CZ-18223 Prague, Czech Republic

Received July 7, 1997. Revised Manuscript Received November 4, 1997<sup>®</sup>

**Abstract:** Distributions of five metal cations ( $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ) and water around a phosphate group with charge  $-1$  compiled from crystal structures showed that these species prefer to bind in sterically well-defined and distinct positions. The differences between the stereochemistry of binding of these metal cations may be related to the different effects they have on thermodynamic properties and conformational equilibria of DNA. All the studied cations prefer asymmetric monodentate positions where they bind to only one of the two charged oxygens (OP) while the bidentate site located symmetrically between the two OP atoms is not occupied at all.  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  have just one binding site,  $\text{Na}^+$  has two sites, and  $\text{K}^+$  and water have three sites. Water distributions from  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ca}^{2+}$  phosphate salts resemble each other but they are modified by the distribution of the accompanying metal. The  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  distributions are spatially most constrained and have the smallest volumes. The distributions of  $\text{K}^+$  and water have up to eight times and that of  $\text{Na}^+$  four times larger volume. The cation and water distributions were analyzed from 18 (9, 9, 6, 12) crystal structures of  $\text{Na}^+$  ( $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Zn}^{2+}$ ) salts of organic phosphates (charge  $-1$ ,  $R$ -factor of 10% or better, deposited in the Cambridge Structural Database) by a Fourier averaging technique described elsewhere (Schneider et al: *Biophys. J.* **1993**, 64, 2291).

## Introduction

Phosphate groups in nucleic acids and lipids, as well as in small nucleotides, are charged under physiological conditions and most of the charge is compensated for by light metal cations such as  $\text{Na}^+$  or  $\text{Mg}^{2+}$ . Alkaline and alkaline earth metals, including the biologically most relevant  $\text{Na}^+$  and  $\text{Mg}^{2+}$ , bind preferably to a phosphate group.<sup>1</sup> Cations reduce the strong repulsion between negatively charged phosphates which would otherwise decrease the stability of the biologically active form of nucleic acids and of densely packed lipids in membranes. Metal–phosphate interactions are, therefore, among the most important forces which model the architecture of nucleic acids (NA) and which influence structure and dynamics of lipid membranes.

Metal cations are known to influence biological function and stability of double helical DNA.<sup>2–4</sup>  $\text{Mg}^{2+}$  is known to increase the DNA melting temperature by stabilizing the duplex structure.<sup>2</sup> DNA denaturates at different temperatures when complexed with different divalent cations,<sup>5</sup> and cations influence the hydrodynamic radius of DNA,<sup>6</sup> and the equilibrium distance

between closely packed DNA molecules.<sup>7</sup> An orderly DNA condensation is necessary for compacting large DNA molecules into cells. A review<sup>8</sup> shows that mechanisms of DNA condensation do not have to be electrostatic and that the condensation is driven as much by kinetic as by thermodynamic factors. According to Bloomfield,<sup>8</sup> the future research should concentrate on a description of sequence effects and the structure of the ion and water atmosphere around NA helices.

There are two general models of metal–NA interactions, ion–atmosphere and site specific binding,<sup>9–11</sup> but neither model can explain all experimental facts.<sup>12</sup> For instance, the melting of the NA regular structure can be explained by the existence of the nonspecific ion atmosphere, but different melting temperatures of different cations<sup>5</sup> suggest at least partially specific binding.

Specific binding of water and of cations plays a large role in interactions between biopolymers.<sup>13</sup> By directly measuring the forces between polyelectrolytic DNA molecules, Rau and Parsegian<sup>14,15</sup> conclude that hydration becomes far more important than electrostatic interactions at distances shorter than 10 Å. The DNA molecule seems to be neutralized far less than

\* To whom correspondence should be addressed.

<sup>®</sup> Abstract published in *Advance ACS Abstracts*, December 15, 1997.

(1) Sigel, H. In *Metal–DNA Chemistry*; Tullius, T. D., Ed.; ACS: Washington, D.C., 1989; Vol. 402, pp 159–204.

(2) Eichhorn, G. L. *Metal Ions in Genetic Information Transfer*; Elsevier: New York, 1981.

(3) Sigel, A.; Sigel, H. *Interactions of Metal Ions with Nucleotides, Nucleic Acids, and Their Constituents*; Marcel Dekker: New York, 1996; Vol. 32.

(4) Cowan, J. A. In *The Biological Chemistry of Magnesium*; Cowan, J. A., Ed.; VCH: New York, 1995; pp 185–209.

(5) Duguid, J. G.; Bloomfield, V. A.; Benevides, J. M.; Thomas, G. J., Jr. *Biophys. J.* **1995**, 69, 2623–2641.

(6) Fujimoto, B. S.; Miller, J. M.; Ribeiro, N. S.; Schurr, J. M. *Biophys. J.* **1994**, 67, 304–308.

(7) Trohalaki, S.; Frisch, H. L.; Lerman, L. S. *Biophys. Chem.* **1991**, 40, 197–205.

(8) Bloomfield, V. A. *Current Opin. Struct. Biol.* **1996**, 6, 334–341.

(9) Felsenfeld, G.; Miles, H. T. *Annu. Rev. Biochem.* **1967**, 36, 407–448.

(10) Anderson, C. F.; Record, M. T., Jr. *Annu. Rev. Biophys. Chem.* **1990**, 19, 423–465.

(11) Manning, G. S. *Q. Rev. Biophys.* **1978**, 11, 179–246.

(12) Porschke, D. In *The Biological Chemistry of Magnesium*; Cowan, J. A., Ed.; VCH: New York, 1995; pp 85–135.

(13) Leikin, S.; Parsegian, V. A.; Rau, D. C. *Annu. Rev. Phys. Chem.* **1993**, 44, 369–395.

(14) Rau, D. C.; Parsegian, V. A. *Biophys. J.* **1992**, 61, 246–259.

(15) Rau, D. C.; Parsegian, V. A. *Biophys. J.* **1992**, 61, 260–271.

expected from ion condensation theory, and the extent of neutralization changes with the kind of cation. Cations therefore change hydration properties of molecular surfaces, and influence the strength of the hydration force and consequently the interaction between biomolecules.

Crystallography, NMR techniques,<sup>16</sup> and computer experiments are particularly effective in resolving the role of specific, i.e. structured, binding of metal cations and water to NA or lipids. A quantum chemical and molecular dynamics study<sup>17</sup> has determined that 80% of Na<sup>+</sup> is directly associated with DNA, and almost exclusively through interactions with phosphates. Na<sup>+</sup> cations have been found approximately equally distributed between the first and second solvation shells. The study has observed the monodentate binding of Na<sup>+</sup> to just one charged phosphate oxygen instead of the sometimes incorrectly predicted bidentate mode. Extensive ab initio calculations of hydrated cations of Mg<sup>2+</sup> and Ca<sup>2+</sup> with dimethyl phosphate<sup>18</sup> have also shown that cations strongly prefer the monodentate position. It has been shown<sup>19</sup> that the stabilization of the monodentate over the bidentate position is due to a mutual interaction between a cation, phosphate, and water molecules. Interestingly, a molecular dynamics study<sup>20</sup> has reported that over half of the Na<sup>+</sup> trajectory has been located in the minor groove of a B-DNA duplex near adenine and thymine bases. Na<sup>+</sup> has also been observed in a similar site in a crystal structure of ApU<sup>21</sup> so that metal cations can intrude into minor grooves. ApU and ApT steps have especially low electrostatic potential in their minor groove parts so that the binding of Na<sup>+</sup> in their minor grooves seems to reflect a sequence effect rather than a general behavior.

Numerous phosphate-containing crystal structures have been determined, including several hundred oligonucleotide structures. Oligonucleotide structures would be the most relevant target for a study of interactions between metals and NA phosphates, but they are solved at such a resolution that metal cations could only rarely be located. Metal coordination in some nucleotide structures has been reviewed by Aoki,<sup>22</sup> who has discussed several types of observed metal-phosphate and metal-base interactions. However, a systematic compilation of cation and water distributions around phosphates has to be based on a larger piece of experimental evidence. Such evidence can be gathered only from crystal structures of organic phosphates with low molecular weight. The first such comparative work aimed at metal-phosphate interactions<sup>23</sup> has shown that metal cations are partially ordered around the anionic phosphinyl group ( $-\text{PO}_2^-$ )<sup>-1</sup> and that they always prefer the asymmetric monodentate position. A limited number of crystal structures available at that time did not allow the study of distributions of specific cations around the whole phosphate group. Recently,

Na<sup>+</sup> distributions have been determined<sup>19</sup> around phosphates with charge  $-1$  and  $-2$ .

The present database study substantially extends the knowledge of the first solvation shell of NA and lipids by determining the spatial distributions of five metal cations, Na<sup>+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Zn<sup>2+</sup>, and water around a negatively charged phosphate group. It demonstrates that a significant fraction of metal cations and water is ordered and prefers to bind in well-defined sites.

## Methods

**Selection of Structures.** The Cambridge Structural Database (CSD)<sup>24</sup> was searched with use of the program QUEST<sup>25</sup> to retrieve structures containing the cations Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, or Zn<sup>2+</sup> and a phosphate fragment  $[\text{X}-\text{O}-(\text{PO}_2)^{-1}-\text{O}-\text{Y}]$ , where X and Y can be either hydrogen(s) or carbon(s), and X can be phosphorus. About 25% of substituents X and Y were hydrogens, 20% aliphatic carbons (not sugars), 16% aromatic carbons, 26% sugars (mostly riboses), and 13% phosphorus from di- and triphosphates. The aliphatic substituents also include frequently occurring enolpyruvates and glycolates. Only structures with crystallographic *R*-factors better than 10% and with average estimated standard deviations of C-C bond lengths no greater than 0.030 Å (a CSD option SIGFLAG 1-3) were accepted.

**Distributions of Metal Cations and Water around the Phosphate Group.** Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Zn<sup>2+</sup> positions closer than 3.00 Å and K<sup>+</sup> and water positions closer than 3.40 Å from the charged phosphate oxygens O1P or O2P were calculated by considering all the symmetry operations of a particular crystal structure.<sup>26</sup> Water molecules were represented by the positions of their oxygen atoms.

Phosphate groups with their associated cations and waters were superimposed over a template phosphate group. The superposition required some preliminary considerations because the  $[-\text{O}-(\text{PO}_2)^{-1}-\text{O}-]$  fragment has two pairs of interchangeable oxygens; two charged and two ester oxygens are potentially indistinguishable. In nucleotide chemistry, the ester oxygens are called O5' and O3', the charged oxygens O1P and O2P. The ester oxygens can be labeled uniquely in nucleotides. For non-nucleotide structures, an oxygen was labeled as O5' when substituted by a hydrogen or by a substituent substantially smaller than a substituent at the other ester oxygen. When substituents at both oxygens were of comparable size a compound was considered twice; the second structure was generated by reflecting the superimposed structure through a symmetry plane defined by the O1P=P=O2P group. The charged oxygens O1P and O2P are harder to label uniquely in a group of chemically varied phosphates so that their labels were permuted in all compounds, including nucleotides. The symmetry related set of structures was generated by applying a symmetry plane O5'-P-O3', which is approximately perpendicular to the O1P=P=O2P plane. All resulting metal and water distributions are therefore symmetrical with respect to the O5'-P-O3' plane.

The metal and water point-like distributions were transformed into pseudoelectron densities by using a previously described procedure.<sup>27,28</sup> The method involves transformation of a 3-D distribution of positions into their densities by Fourier averaging with use of the program X-Plor<sup>29</sup>. Density maps were displayed and peaks fitted with the program O<sup>30</sup>. The highest densities in the map represent sites of preferential cation or water binding.

(16) Sletten, E.; Froystein, N. A. In *Interactions of Metal Ions with Nucleotides, Nucleic Acids, and Their Constituents*; Sigel, A., Ed.; Marcel Dekker: New York, 1996; Vol. 32, pp 397-418.

(17) Laughton, C. A.; Luque, F. J.; Orozco, M. *J. Phys. Chem.* **1995**, *99*, 11591-11599.

(18) Deerfield, D. W., II; Pedersen, L. G. *J. Biomol. Struct. Dyn.* **1995**, *13*, 167-179.

(19) Schneider, B.; Kabeláč, M.; Hobza, P. *J. Am. Chem. Soc.* **1996**, *118*, 12207-12217.

(20) Young, M. A.; Jayaram, B.; Beveridge, D. L. *J. Am. Chem. Soc.* **1997**, *119*, 59-69.

(21) Seeman, N. C.; Rosenberg, J. M.; Suddath, F. L.; Kim, J. J. P.; Rich, A. *J. Mol. Biol.* **1976**, *104*, 109-144.

(22) Aoki, K. In *Interactions of Metal Ions with Nucleotides, Nucleic Acids, and Their Constituents*; Sigel, A., Ed.; Marcel Dekker: New York, 1996; Vol. 32, pp 91-134.

(23) Alexander, R. S.; Kanyo, Y. F.; Chirlian, L. E.; Christianson, D. W. *J. Am. Chem. Soc.* **1990**, *112*, 933-937.

(24) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rodgers, J. R.; Watson, D. G. *Acta Crystallogr.* **1979**, *B35*, 2331-2339.

(25) Allen, F. H.; Davies, J. E.; Galloy, J. J.; Johnson, O.; Kennard, O.; Macrae, C. F.; Mitchell, E. M.; Mitchell, G. F.; Smith, J. M.; Watson, D. G. *J. Chem. Inf. Comput. Sci.* **1991**, *31*, 187-204.

(26) Cohen, D.; Vadaparty, K.; Dickinson, B. *Efficient algorithms for distance queries in macromolecular structure databases*; University of Pittsburgh: Pittsburgh, 1995.

(27) Schneider, B.; Cohen, D. M.; Schleifer, L.; Srinivasan, A. R.; Olson, W. K.; Berman, H. M. *Biophys. J.* **1993**, *65*, 2291-2303.

(28) Schneider, B.; Berman, H. M. *Biophys. J.* **1995**, *69*, 2661-2669.

(29) Brünger, A. T. *X-PLOR Manual*, version 3.1; Yale University: New Haven, CT, 1992.

**Table 1.** Statistics of Contacts between Metal Cations, Phosphates, and Water in Crystals

cation	total number Analyzed				contacts from cation to <sup>c</sup>		
	structures	cations <sup>a</sup>	PO <sub>4</sub> <sup>-</sup> cations <sup>b</sup>	waters	PO <sub>4</sub> <sup>-</sup>	H <sub>2</sub> O	other
Na <sup>+</sup>	18	33	26	31	47	58	52
Mg <sup>2+</sup>	9	16	14	35	30	29	27
K <sup>+</sup>	9	17	14	9	39	12	39
Ca <sup>2+</sup>	6	7	7	34	22	31	13
Zn <sup>2+</sup>	12	16	13	37	26	10	38

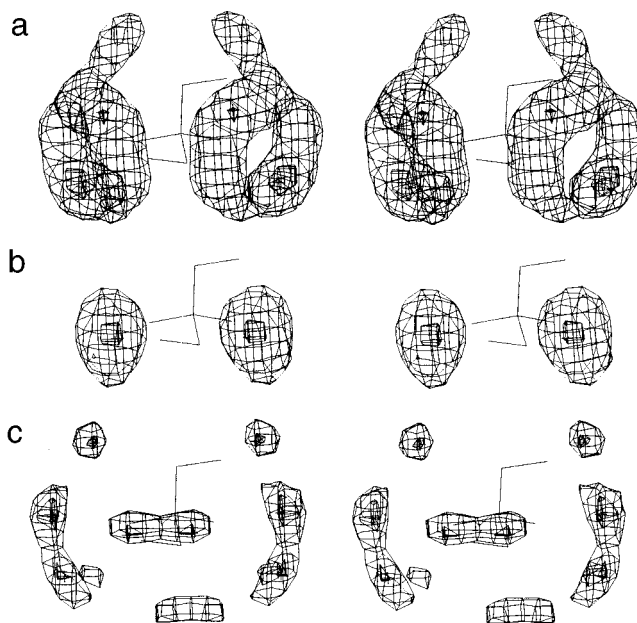
<sup>a</sup> Number of cations of a given type. <sup>b</sup> Number of cations coordinated by a phosphate. Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Zn<sup>2+</sup> are called coordinated by a phosphate when no further than 3.00 Å from any of the phosphate atoms and K<sup>+</sup> and water when no further than 3.40 Å. Of 33 Na<sup>+</sup> cations, seven (33 - 26) are not directly coordinated by a phosphate group. <sup>c</sup> Number of contacts with distances less than 3.00 Å (Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Zn<sup>2+</sup>) or 3.40 Å (K<sup>+</sup>) between a cation and atoms of a specified group. Note the difference between the number of analyzed cations and the number of their contacts. For instance, one Na<sup>+</sup> can make two contacts to phosphate oxygens, one contact to a water molecule, and two contacts to other nucleophilic groups.

## Results and Discussion

**Statistics of Contacts.** The numbers of analyzed structures and of cations they contain are listed in Table 1. The analysis can be considered most reliable for Na<sup>+</sup>, while it is tentative for Ca<sup>2+</sup>. There are two di- or triphosphates among Na<sup>+</sup> and K<sup>+</sup> structures and one among Mg<sup>2+</sup> and Zn<sup>2+</sup> structures—totally six structures. Table 1 shows that crystals containing divalent cations tend to be hydrated more than crystals of monovalent cations. In most cases, metals interact directly with the phosphate (column “PO<sub>4</sub><sup>-</sup> cations” in Table 1), but a significant fraction of about 25% of monovalent cations Na<sup>+</sup> and K<sup>+</sup> is located in the second solvation shell. With one exception, all cations located in the second shell are coordinated to the phosphate via a water molecule forming so-called outer-sphere complexes. In solution, the proportion of outer complexes most probably exceeds the 25% observed in the crystal phase.<sup>4,12</sup> The only transition metal in the sample, Zn<sup>2+</sup>, has a larger affinity to organic substituents than to phosphate or water (last three columns in Table 1)—the environment of all other cations is primarily determined by electrostatic and hydration interactions. Contacts to water prevail with Ca<sup>2+</sup> while those to non-water prevail with K<sup>+</sup> and Zn<sup>2+</sup>.

**Distributions of Metal Cations.** Distributions of Na<sup>+</sup>, Mg<sup>2+</sup>, and K<sup>+</sup> are plotted in Figure 1a–c. The metal distributions point toward the viewer and the charged oxygens are on the left and right. Note that only the left or right half of the distributions is independent, while the other half is related by the O5'–P–O3' symmetry plane. The preferred binding sites are indicated by crosses.

The distributions clearly demonstrate that cation positions around the –1 phosphate group are not random and that they are localized in well-defined sites. All the studied cations bind in asymmetric monodentate positions inside the sector limited by the phosphate atoms OP=P=OP where they are attached to only one of the two charged oxygens. This asymmetric monodentate position is the only position for Mg<sup>2+</sup> and Zn<sup>2+</sup> while other cations can also be found elsewhere: outside the OP=P=OP sector (Na<sup>+</sup>, K<sup>+</sup>) or trapped between the charged oxygen OP and the ester oxygen O3' (K<sup>+</sup>). It should be stressed that the bidentate site located symmetrically between the two OP atoms is not occupied by either metal at all.

**Figure 1.** A stereoview of distributions of metal cations around a negatively charged phosphate group for Na<sup>+</sup> (a), Mg<sup>2+</sup> (b), and K<sup>+</sup> (c).**Table 2.** The Binding Sites of Metal Cations and of Water Molecules around a Negatively charged phosphate group

metal cation M	distance <sup>a</sup> M···OP	angle <sup>a</sup> M···OP=P	torsion <sup>a</sup> M···OP=P=OP	type of site <sup>b</sup>	rel density <sup>c</sup>
Na <sup>+</sup>	2.4	130	–50	IN	8
Na <sup>+</sup>	2.3	125	130	OUT	9
Mg <sup>2+</sup>	1.9	140	0	IN	17
K <sup>+</sup>	2.8	115	–10	IN	6
K <sup>+</sup>	2.4	165	–130	OUT	7
K <sup>+</sup>	2.9 <sup>d</sup>	95	–140	ESTER	6
Zn <sup>2+</sup>	1.8	160	–45	IN	14

water W <sub>M</sub> <sup>e</sup>	distance <sup>a</sup> W <sub>M</sub> ···OP	angle <sup>a</sup> W <sub>M</sub> ···OP=P	torsion <sup>a</sup> W <sub>M</sub> ···OP=P=OP	type of site <sup>b</sup>	rel density <sup>c</sup>
W <sub>Na</sub> <sup>+</sup>	2.7	125	0	IN	8
W <sub>Na</sub> <sup>+</sup>	2.7	145	180	OUT	7
W <sub>Na</sub> <sup>+</sup>	2.6	125	110	OUT	6
W <sub>Mg</sub> <sup>2+</sup>	3.1	95	–20	BRIDGE	8
W <sub>Mg</sub> <sup>2+</sup>	2.7	155	180	OUT	7
W <sub>Mg</sub> <sup>2+</sup>	2.7	120	–150	OUT	9

<sup>a</sup> Distances are in angstroms and angles in degrees. <sup>b</sup> “IN” indicates a site located inside and “OUT” indicates a site located outside the OP=P=OP sector but always attached to one charged oxygen OP, “ESTER” indicates attachment primarily to the ester oxygen O3', and “BRIDGE” means a position bridging both charged oxygens. <sup>c</sup> The relative density indicates how many times higher the density of a binding site is than the estimated standard deviation of the density map. <sup>d</sup> The distance to the esterified oxygen O3' is 2.6 Å. <sup>e</sup> A subscript indicates in what phosphate salt a water distribution was analyzed.

Mg<sup>2+</sup> and Zn<sup>2+</sup> have just one binding site around each OP and their distributions are the tightest of all the metals (Figure 1b for Mg<sup>2+</sup>). The distributions and binding sites of Mg<sup>2+</sup> and Zn<sup>2+</sup> are very similar, Zn<sup>2+</sup> is closer to the P=OP line. The Na<sup>+</sup> distribution (Figure 1a) contrasts with the sharply focused Mg<sup>2+</sup>/Zn<sup>2+</sup> distributions. Na<sup>+</sup> can bind in a wide yet well-defined ring-shaped tube that is outlined by a constant Na<sup>+</sup>···OP distance of 2.4 Å and an Na<sup>+</sup>···OP=P angle of 125°. Two binding sites were located inside the tube (Table 2). Both sites lie outside the OP=P=OP plane, one inside and one outside the OP=P=OP sector. Despite the fact that density variations inside the tube are small, the fitted positions most probably correctly represent Na<sup>+</sup> binding preferences because several

density maps based on different sets of  $\text{Na}^+$  structures repeatedly showed similar positions. The positions reported here agree within 0.5 Å with the positions determined previously<sup>19</sup> on a smaller set of sodium phosphate salts.

Two main  $\text{K}^+$  sites lie close to the  $\text{OP}=\text{P}=\text{OP}$  plane, one inside and one outside the  $\text{OP}=\text{P}=\text{OP}$  sector (Figure 1c). One weaker site could be fitted close to the  $\text{Na}^+$  "OUT" position, but it is not listed in Table 2.

The tentative  $\text{Ca}^{2+}$  distribution can be regarded as an intermediate between the focused  $\text{Mg}^{2+}$  and more dispersed  $\text{Na}^+/\text{K}^+$  distributions. The  $\text{Ca}^{2+}$  sites are localized between the  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  sites and the  $\text{Na}^+$  "OUT" site. The observation that  $\text{Ca}^{2+}$  has probably more than one binding site and is less restrictive in its binding than  $\text{Mg}^{2+}$  agrees with *ab initio* calculations by Deerfield and Pedersen.<sup>18</sup> Also Laing and Draper<sup>31</sup> observed that  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  bind differently to the phosphate group.

The distances between metal cations and charged phosphate oxygens found here are generally within 0.1 Å from the cation–water internuclear distances reviewed by Marcus.<sup>32</sup> All monovalent metals show surprisingly small differences between distances to the charged phosphate and uncharged water oxygens. The differences are larger for divalent cations with the largest difference of 0.3 Å for  $\text{Zn}^{2+}$ . Probably only stronger electrostatic interactions between dications and phosphates can shorten metal–oxygen distances.

Asymmetric monodentate binding of metal cations to just one charged oxygen has also been identified as the most prevailing binding mode for the anionic phosphinyl group  $(-\text{PO}_2^-)^{-1}$  by Alexander et al.<sup>23</sup> and for a carboxylic group.<sup>33</sup> In contrast to the phosphate, carboxyls bind about 15% of cations to the symmetric bidentate position bound to both partially charged carboxylic oxygens. Only  $\text{Na}^+$  has been found to bind out of the  $\text{COO}^-$  plane, which is in general agreement with our observation of nonplanar  $\text{Na}^+$  positions. For both carboxylates and phosphates,  $\text{Mg}^{2+}$  has tighter distributions than  $\text{Na}^+$ .

**How General Are the Distributions?** Do the calculated distributions represent the general behavior of the studied cations or are they just a consequence of a particular set of structures from which they were derived? An answer could be obtained by calculating distributions for a particular conformation of chemically homogeneous phosphates, for instance for  $\text{Na}^+$  salts of nucleotides in the *-sc/-sc* conformation. Because such a highly homogeneous group of crystallized phosphates is not available, one has to settle for less ultimate yet generally valid arguments. Most importantly, a majority of cations bind to the charged oxygens far apart ( $\sim 5$  Å) from substituents of the ester oxygens. Metals such as  $\text{Mg}^{2+}$  or  $\text{Zn}^{2+}$  with tight distributions are apparently influenced less than more diffuse  $\text{Na}^+$  or  $\text{K}^+$ .

The relationship between phosphate substitution and a cation distribution was partially analyzed by looking at  $\text{Na}^+$  phosphates with the  $\text{O5}'$  oxygen substituted exclusively by hydrogen rather than by carbon. A number of hydrogen phosphates in the sample of sodium salts allowed us to calculate a map of the  $\text{Na}^+$  distribution around monohydrogen phosphates. The map was very similar to the map calculated for all  $\text{Na}^+$ ; the area around the charged oxygens remained virtually unchanged and the corresponding binding sites were fitted within 0.3 Å from each other. The carbon to hydrogen substitution at  $\text{O5}'$  therefore does not influence the  $\text{Na}^+$  distribution.

A substitution effect can, however, occur under more specific circumstances. The lobe of  $\text{Na}^+$  density pointing toward the oxygen  $\text{O3}'$  on the top of Figure 1a originates from cations bound to four  $-(\text{PO})_2^- - \text{O} - \text{C} - \text{COO}^-$  fragments and one fragment where the  $-\text{COO}^-$  group is replaced by a sugar moiety. These cations are held between the phosphate oxygens  $\text{OP}$  and  $\text{O3}'$ , and an oxygen of the near electronegative group. The corresponding metal site is even more pronounced in  $\text{K}^+$  (density on the top of Figure 1c) than in  $\text{Na}^+$  phosphates because the  $-(\text{PO})_2^- - \text{O} - \text{C} - \text{COO}^-$  motif is observed in a larger fraction of  $\text{K}^+$  salts. It should be stressed that no  $\text{Mg}^{2+}$  cations were observed in a similar position even when they were coordinated by carboxy groups such as  $\text{Na}^+$  and  $\text{K}^+$  and that no cation is in a similar position in di- or triphosphates.

Metal distributions from di- and triphosphate structures could not be analyzed separately because of their low number, but excluding them from the set of all  $\text{Na}^+$  structures did not substantially change the original distribution and the corresponding binding sites were in virtually the same positions. Changes were observed only in the area outside of the  $\text{OP}=\text{P}=\text{OP}$  sector where exclusion of diphosphates decreased the density.

In di- and triphosphates one metal cation is often coordinated by two or even more phosphate groups. One referee pointed out that such multiple coordinations could skew the preferred metal positions around the single phosphate group. To test whether particular features of metal distributions, especially the existence of the "OUT" sites for  $\text{Na}^+$  and  $\text{K}^+$ , are a consequence of packing arrangements around cations, we analyzed how metals are coordinated in crystals. Of 26  $\text{Na}^+$  cations coordinated directly by a phosphate group, 12 are contacted by two and 9 by three or more phosphate groups.  $\text{K}^+$  and  $\text{Mg}^{2+}$  have similar distributions: of 14  $\text{K}^+$  in contact with a phosphate, 4 are coordinated by two and 8 by more than two phosphates, of 14  $\text{Mg}^{2+}$ , 2 are coordinated by two phosphates and 7 by more than two. Crystal packing imposes multiple phosphate coordination on  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{2+}$  cations with similar frequency so that this effect cannot by itself explain the existence of fundamentally different patterns of  $\text{Na}^+/\text{K}^+$  and  $\text{Mg}^{2+}$  distributions. The available data suggest that parts of  $\text{Na}^+$  and  $\text{K}^+$  distributions located outside of the  $\text{OP}=\text{P}=\text{OP}$  sector can be explained by neither substitution nor packing effects and that  $\text{Na}^+$  and  $\text{K}^+$  are less restrictive in their binding than  $\text{Mg}^{2+}$ .

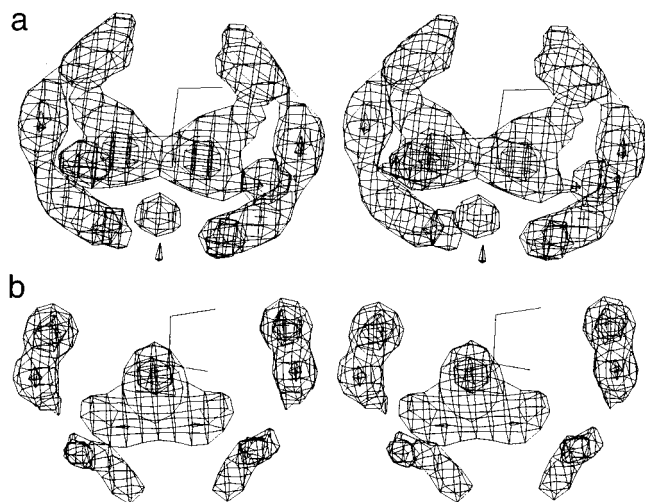
**Distributions of Water Molecules.** Water distributions are more diffuse than distributions of metal cations and there are always three hydration sites hydrogen bonded to one charged oxygen. Even when water distributions do not generate such a regular toroidal density as  $\text{Na}^+$ , their character resembles the  $\text{Na}^+$  distribution (Figure 2a,b). Water molecules bind in a relatively large area around the phosphate and the actual binding sites are determined by site(s) preferred by accompanying metals. Hydration sites are located at  $\text{W} \cdots \text{OP}$  distances of about 2.7 Å and  $\text{W} \cdots \text{OP}=\text{P}$  angles between 100° and 150° (Table 2), suggesting strong hydrogen bonding. No water site is attached to ester oxygens.

In the case of the divalent metals  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ , and possibly  $\text{Ca}^{2+}$ , one water is shared by both charged oxygens so that there are five waters per phosphate.  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ , metals with just one binding position and tight distributions, do not alternate with their hydration sites. On the other side, the  $\text{Na}^+$  site and the corresponding hydration site, both labeled "OUT" in Table 2, and having the torsion of about 120°, are close to each other, and can be occupied only by  $\text{Na}^+$  or by water at any given

(31) Laing, L. G.; Draper, D. E. *J. Mol. Biol.* **1994**, 237, 560–576.

(32) Marcus, Y. *Chem. Rev.* **1988**, 88, 1475–1498.

(33) Carrell, C. J.; Carrell, H. L.; Erlebach, J.; Glusker, J. P. *J. Am. Chem. Soc.* **1988**, 110, 8651–8656.



**Figure 2.** A stereoview of water distributions around a negatively charged phosphate group in  $\text{Na}^+$  salts (a) and in  $\text{Mg}^{2+}$  salts (b).

time. No results can be presented for  $\text{K}^+$  because potassium salts are hydrated to a limited extent (Table 1).

Three water molecules per charged oxygen were predicted to saturate the first hydration shell of the phosphate group in papers from Pullmans' group<sup>34</sup> and later confirmed by crystallographic observations on oligonucleotides.<sup>35</sup> The arrangement of water molecules around the phosphate is sometimes called "a cone of hydration". Our findings from small molecule crystals are in agreement with water distributions observed in crystal structures of oligonucleotides.<sup>36</sup>

**Volumes Accessible to Metals and Water.** The density distributions (Figures 1 and 2) offer more than just qualitative information about metal or water distributions and about the location of binding sites. They limit the space available to a metal cation or water. A volume confined within a certain level of density is a measure of the number of states available to a species and relates to its conformational entropy. The volumes of distributions were estimated as the numbers of points from a regular grid which had densities higher than a certain threshold. Selection of the threshold is not critical because not absolute values but fractions of volumes are compared between different species. Results discussed here are based on volumes confined by densities higher than 5/16 and 2/16 of the highest peak.

Because  $\text{Mg}^{2+}$  has the tightest distribution its volume was used as a gauge for volumes of all other species. The difference is largest for water, which can occupy five to eight times more space, and for  $\text{K}^+$ , which can occupy space eight times larger than  $\text{Mg}^{2+}$ . The  $\text{Na}^+$  distribution is four times and the  $\text{Ca}^{2+}$

distribution almost three times more dispersed than the  $\text{Mg}^{2+}$  distribution while  $\text{Zn}^{2+}$  occupies only 50% more space.

The metals which are least spatially constrained in their binding,  $\text{K}^+$  and  $\text{Na}^+$ , can fluctuate more freely and have a higher conformational entropy than relatively tightly constrained  $\text{Mg}^{2+}$ . Extremely different volumes of distributions and the existence of multiple versus single binding sites also has an energetic connotation. It is conceivable that an interacting molecule binds to the phosphate group inside the  $\text{OP}=\text{P}=\text{OP}$  sector, thus blocking the only  $\text{Mg}^{2+}$  binding site. At the same time, monovalent cations  $\text{K}^+$  and  $\text{Na}^+$  can still bind in their energetically preferred positions. A quantitative estimate of such an effect in terms of free energy is, however, hard. We do not know how to scale the calculated difference between accessible volumes of two cations into the difference of their entropies. Further, metals can bind to phosphates not only directly ("inner-sphere binding") but also via water ("outer-sphere binding"), and concentration of these outer complexes in solution is estimated to be as high as 50% of the bound metal.<sup>12</sup>

The spatially enclosed metal and water distributions demonstrate that these species can bind to specific sites at the DNA or lipid surface and their release from the surface during interaction with another biomolecule can be a source of the attractive entropic force.<sup>15</sup> The observed variations in the spatial distributions of different metal cations support the view that interactions between biomolecules depend on a type of cation and that they can be controlled by reconfiguring the structured solvent between macromolecular surfaces.<sup>14</sup>

## Conclusions

$\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Zn}^{2+}$  have spatially localized distributions around the  $-1$  phosphate group with one ( $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ ), two ( $\text{Na}^+$ ), or three ( $\text{K}^+$ ) binding sites around each charged oxygen. Both distributions and binding sites are distinct for the above metals. The distributions are primarily determined by metal–phosphate interactions and the stereochemistry of the interaction is only modified by phosphate substituents and crystal packing. The water distributions are more diffuse than the metal distributions and, similarly to  $\text{Na}^+$ , encircle each charged phosphate oxygen. There are three hydration sites around each charged phosphate oxygen, but their actual positions vary according to a present metal cation.

**Acknowledgment.** This work was supported by grants from the Grant Agency of the Czech Republic to B.S. (204/95/0036) and for purchase of the CSD (203/96/0111).

**Supporting Information Available:** A bibliography of the analyzed structures, an extended version of Table 2, five stereo figures of distributions of  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Zn}^{2+}$ , and four figures of water distributions in  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Zn}^{2+}$  phosphate salts in the PostScript format (16 pages). See any current masthead page for ordering and Internet access instructions.

JA972237+

(34) Langlet, J.; Claverie, P.; Pullman, B.; Piazzola, D. *Int. J. Quantum Chem.* **1979**, 6, 409–437.

(35) Westhof, E. *Water and Biological Macromolecules*; CRC: Boca Raton, 1993.

(36) Schneider, B.; Patel, K.; Berman, H. M. *Progr. Biophys. Mol. Biol.* **1996**, 65, 70–70.