Inorg. Chem. **2005**, *44*, 831–833

Inorganic Chemistry

Communication

Anions or Cations: Who Is in Charge of Inhibiting the Nickel(II) Promoted B- to Z-DNA Transition?

Bernhard Spingler*

University of Zürich, Institute of Inorganic Chemistry, Winterthurerstr. 190,
CH-8057 Zürich, Switzerland

Received September 3, 2004

Various weakly binding cations and anions were studied at a concentration of 10 mM to ascertain their interaction with the nickel(II) promoted B- to Z-DNA transition of poly d(GC). These salts were ranked according to the decreasing amounts of nickel needed for the B- to Z-DNA transition and provided the following order: NaCl $\approx$ Me_4NCl > LiCl $\gg$ $MgCl_2$ > no salt > $NaBF_4$ $\approx$ $NaNO_3$ $\approx$ $NaClO_4$. Remarkably, it was found that going from sodium nitrate to sodium chloride increased the necessary amount of nickel to induce the transition to the left-handed helix of poly d(GC) by a factor of 10. This dramatic effect cannot be explained by the binding constant of nickel(II) to chloride to form the monocationic complex. We believe that this is the first reported example of the role of chloride anions, which appear to modulate the interaction of nickel(II) ions with the polyanionic DNA.

It is widely accepted that alkali and alkaline earth ions compensate the negative charge of the phosphates in the backbone of DNA and RNA in vivo.[1] The distribution of mono- and divalent ions around the right-handed B-DNA has been studied, but only in theory. The anion density in the DNA grooves and on the phosphates was found to be small and unstructured.[2] The binding of most transition metals to DNA was found to be much more defined. Several X-ray structures of short double stranded DNA have been elucidated together with nickel(II).[3] They showed that the nickel ions were coordinated to N7 of guanosine, via an inner sphere mode. Sigel et al. determined the logarithmic binding constant of Ni^{2+} to GMP^{2-} to have a pK value of 3.13 ± 0.03, and that the complex exists mostly as an intramolecular chelate ring.[4] Furthermore, nickel(II) was found to preferentially bind to GMP compared with AMP. Several investigations have shown that, in the case of double stranded DNA, nickel(II) can bind to two positions on the DNA, most probably to N7 of guanosine and to the phosphate group.[5–7]

Table 1. Sodium Chloride Dependence of the Nickel(II) Promoted B- to Z-DNA Transition of Poly d(GC)

[NaCl]/mM	equiv of Ni/DNA phosphates needed for the B- to Z-DNA midpoint
0	0.62[11]
0	0.4–0.9[7]
7	3–6[7]
10	9[12]
97	no transition[7]

The existence of left-handed Z-DNA was discovered when poly d(GC) was in the presence of very high concentrations of salts, such as sodium (>2.5 M) or magnesium (>0.7 M) chloride.[8] There is continuing interest in deciphering the role Z-DNA plays in vivo.[9] Furthermore, recent publications were concerned with the design and use of new artificial ligands, which promote the formation of Z-DNA.[10]

Low concentrations of transition metals, as their aqua ions, were also able to convert pyrimidine–purine alternating DNA sequences from the B- to the Z-DNA conformation.[7,13] More interestingly, this process was highly disfavored in the presence of small amounts of sodium chloride.[7,12,14] Table 1

---

* E-mail: spingler@aci.unizh.ch.

(1) Black, C. B.; Huang, H. W.; Cowan, J. A. *Coord. Chem. Rev.* **1994**, *135*, 165–202.

(2) Allahyarov, E.; Löwen, H.; Gompper, G. *Phys. Rev. E* **2003**, *68* article number 061903.

(3) Abrescia, N. G. A.; Huynh-Dinh, T.; Subirana, J. A. *J. Biol. Inorg. Chem.* **2002**, *7*, 195–199. Abrescia, N. G. A.; Malinina, L.; Subirana, J. A. *J. Mol. Biol.* **1999**, *294*, 657–666. Abrescia, N. G. A.; Malinina, L.; Fernandez, L. G.; Huynh-Dinh, T.; Neidle, S.; Subirana, J. A. *Nucleic Acids Res.* **1999**, *27*, 1593–1599.

(4) Sigel, H.; Massoud, S. S.; Corfu, N. A. *J. Am. Chem. Soc.* **1994**, *116*, 2958–2971.

(5) Kasprzak, K. S.; Waalkes, M. P.; Poirier, L. A. *Toxicol. Appl. Pharm.* **1986**, *82*, 336–343. Datta, A. K.; Riggs, C. W.; Fivash, M. J.; Kasprzak, K. S. *Chem.-Biol. Interact.* **1991**, *79*, 323–334.

(6) Kankia, B. I. *Nucleic Acids Res.* **2000**, *28*, 911–916.

(7) Schoenknecht, T.; Diebler, H. *J. Inorg. Biochem.* **1993**, *50*, 283–298.

(8) Pohl, F. M.; Jovin, T. M. *J. Mol. Biol.* **1972**, *67*, 375–396.

(9) Rothenburg, S.; Koch-Nolte, F.; Haag, F. *Immunol. Rev.* **2001**, *184*, 286–298. Rich, A.; Zhang, S. *Nat. Rev. Genet.* **2003**, *4*, 566–572.

(10) Qu, X.; Trent, J. O.; Fokt, I.; Priebe, W.; Chaires, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12032–12037. Xu, Y.; Zhang, Y. X.; Sugiyama, H.; Umano, T.; Osuga, H.; Tanaka, K. *J. Am. Chem. Soc.* **2004**, *126*, 6566–6567.

(11) Shih, H.-C.; Tang, N.; Burrows, C. J.; Rokita, S. E. *J. Am. Chem. Soc.* **1998**, *120*, 3284–3288.

(12) van de Sande, J. H.; McIntosh, L. P.; Jovin, T. M. *EMBO J.* **1982**, *1*, 777–782.

(13) Fazakerley, G. V. *Nucleic Acids Res.* **1984**, *12*, 3643–3648. Middendorf, S. M.; Diebler, H. *Ber. Bunsen-Ges. Phys. Chem.* **1996**, *100*, 641–647.

(14) Rossetto, F. E.; Nieboer, E. *J. Inorg. Biochem.* **1994**, *54*, 167–186.

10.1021/ic048760o CCC: $30.25

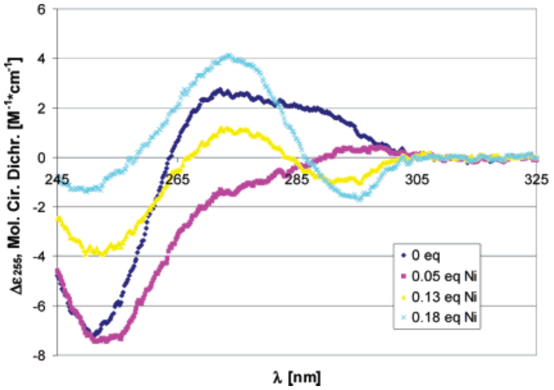

Figure 1. Selected CD spectra of the titration of poly d(GC) with various equivalents of nickel nitrate versus DNA phosphate in 1 mM sodium cacodylate buffer (pH = 7) and 10 mM $NaClO_4$.

summarizes this general trend for the nickel scenario. It can be seen that, in the absence of sodium chloride, the B- to Z-DNA transition took place, when there was about one nickel(II) present per base pair. Low millimolar concentrations of NaCl required 10 times more nickel for the formation of Z-DNA, and interestingly, at a concentration of about 100 mM NaCl, no Z-DNA could be detected.

The logarithmic binding constant log β_1 of Ni^{2+} and Cl^- to form $[NiCl]^+$ is -0.8.[15] This means that, in a 10 mM NaCl solution having a Ni^{2+} concentration of 0.1 mM,[16] only 0.2% of all the nickel ions were forming the monocationic complex $[NiCl]^+$. Therefore, it was not clear a priori whether the sodium cation, the chloride anion, or both were responsible for inhibiting the nickel ions in transforming poly d(GC) from the right- to the left-handed helix.

In this Communication, we present a study of the influence of anions and cations, which weakly bind to DNA and nickel. More importantly, we shed new light on the role of anions in DNA chemistry. Poly d(GC) was dissolved in 1 mM sodium cacodylate buffer (pH = 7) together with the designated salts. The DNA was then titrated with a 1 or 10 mM solution of nickel nitrate dissolved in the same buffer. After each addition, the solution was heated in an external water bath to 60 °C for 5 min, in order to establish the thermodynamic equilibrium,[12] before cooling down to room temperature (see Supporting Information).

A variety of salts, at a concentration of 10 mM, were tested for their role in the nickel promoted B- to Z-DNA transition, such as NaCl, $NaBF_4$, $NaNO_3$, $NaClO_4$, Me_4NCl, LiCl, and $MgCl_2$. The $MgCl_2$ was used at a concentration of 5 mM in order to have the same chloride concentration as in the case of the other chloride containing solutions. For comparison, a control solution, containing only the buffer, was also studied. Representative measurements are shown in Figures 1 and 2.

The titrations were monitored at 255 nm (Figure 3), where a negative CD signal of $\Delta\epsilon \approx -6$ is typical for B-DNA, while slightly positive $\Delta\epsilon$ values are indicative of the Z-DNA form.

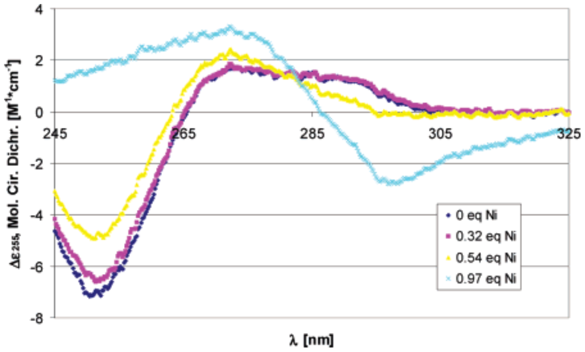

Figure 2. Selected CD spectra of the titration of poly d(GC) with various equivalents of nickel nitrate versus DNA phosphate in 1 mM sodium cacodylate buffer (pH = 7) and 5 mM $MgCl_2$.

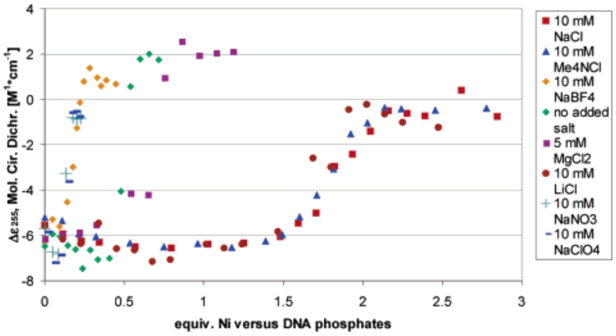

Figure 3. The influence of salt on the nickel promoted B- to Z-DNA transition. All titrations were conducted in 1 mM sodium cacodylate buffer (pH = 7), along with the salt mentioned in the legend.

Table 2. Salt Promoted B- to Z-DNA Transition of Poly d(GC) Compared with Nickel Induced B- to Z-DNA Transition at 10 mM Salt Concentrations (5 mM for $MgCl_2$)

salt	midpoint of B- to Z-DNA induced by the salt alone	equiv of Ni^{2+} needed for the midpoint of the B- to Z-DNA transition
NaCl	2.4 M[8]	1.75
Me_4NCl	3.2 M[17]	1.75
LiCl	>5 M at 67° C[18]	1.7
$MgCl_2$	0.7 M[8]	0.7
no salt	no transition	0.55
$NaBF_4$	unknown	0.2
$NaNO_3$	lower than 2.4 M[19]	0.2
$NaClO_4$	1.5 M[8]	0.15

The above-mentioned salt solutions were ranked according to the decreasing amounts of nickel needed for the B- to Z-DNA transition and provided the following order: NaCl $\approx$ Me_4NCl > LiCl $\gg$ $MgCl_2$ > no salt > $NaBF_4$ $\approx$ $NaNO_3$ $\approx$ $NaClO_4$. This series was compared with the required salt concentrations, that converted the B- to Z-DNA without nickel (Table 2).

In the process of analyzing the results, we attempted to differentiate between the influences of the cations and the anions. All the studied cations favored the formation of Z-DNA. This was in contrast to the anions, which were found to have either neutral to strongly inhibiting effects. All the chloride salts hinder the B- to Z-DNA transition compared with the salt free reaction. $MgCl_2$ had the least hindering effect on the equilibrium. This can be explained by the fact that $MgCl_2$ alone was only needed at $^1/_3$ of the corresponding concentration of sodium chloride for the formation of Z-DNA to occur. In addition, the CD spectra of the titration of $MgCl_2$

---

(15) Iuliano, M.; Porto, R.; Vasca, E. *Ann. Chim. (Rome)* **1989**, *79*, 439–454.

(16) At this 0.1 mM concentration of Ni^{2+} in the absence of sodium chloride, the B- to Z-DNA transition of poly d(GC) is already over.

solution provided evidence for a different reaction pathway (see below).

Remarkably, it was found that going from sodium nitrate to sodium chloride, both at the same 10 mM concentration, increased the amount of nickel needed to induce the left-handed form of poly d(GC)$_n$ by a factor of 10. The weak binding constants of chloride to nickel to form either the 1:1 adduct or the 2:1 complex do not explain this observed behavior, as described above. In all the examples studied, with the exception of the magnesium, nickel was shown to bind first to B-DNA. This was proven spectroscopically by the decrease in the positive CD band in the range 270−290 nm, which has been reported in the transition metal promoted transition of B- to Z-DNA[6] (see Figure 1). This decrease in the CD signal did not occur in the solution with the magnesium chloride (see Figure 2). Thus, the B- to Z-DNA transition occurred without showing the intermediate CD spectrum of a nickel(II) bound to B-DNA. Obviously, the magnesium(II) displaced the nickel(II) from a common binding place on the B-DNA, which most likely was in proximity to the phosphate group. In order to estimate the binding of Mg^{2+} to poly d(GC), we looked at the closely related situation of magnesium(II) binding to the mononucleotides GMP and CMP, which were determined to have a logarithmic binding constant of 1.81 ± 0.04[20] and 1.93 ± 0.1,[21] respectively. Assuming that one can apply these constants also to describe the binding of Mg^{2+} to poly d(GC), and using the average of these two binding constants, 26% of the phosphate groups of poly d(GC) are bound to magnesium. This seems to be sufficient to prevent the nickel ions from binding to the phosphodiester groups. Nevertheless, magnesium and nickel synergistically contribute to the conversion of B- to Z-DNA.

These experiments clearly demonstrate that chloride ions exclusively inhibit the nickel promoted B- to Z-DNA transition in a very efficient way. It is important to note that no structure of Z-DNA with nickel(II) can be found in the nucleic database (NDB). Taking the known binding constants of chloride to nickel(II) into account, it is not possible that the chloride ions can deactivate free aquated nickel(II) by coordination. An alternative explanation is that only the nickel(II) bound to DNA was deactivated by chloride ions (Figure 4). The B- to Z-DNA transition of poly d(G 5-methyl-C) was described by two different mechanisms:[22] At low salt concentrations, melting of the two strands at the DNA termini induces the transition. At higher salt concentrations, the initial transition was predicted to happen within the double strand. At this time, it is not clear how either of these two mechanisms is inhibited by the ternary arrangement of nickel and chloride ions coordinating to DNA.

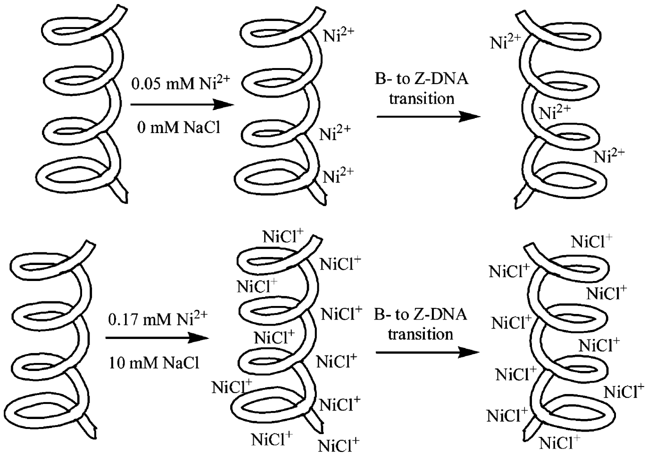

Figure 4. Possible mechanism by which the chloride ions might inhibit the nickel promoted B- to Z-DNA transition.

We believe that this is the first report about the role of chloride anions, which modulate the interaction of nickel ions with the polyanionic DNA. In studying the behavior of DNA, it is clear that the cations are essential to balance the negative charge of the phosphate groups. However, this should not lead one to oversimplify the notion that weakly binding anions play no role in the chemistry of transition metals interacting with nucleobases.

Acknowledgment. Support by the Swiss National Science Foundation and the University of Zürich is gratefully acknowledged. I thank Professors Roger Alberto, Richard H. Fish, Helmut Sigel, and Roland Sigel for stimulating discussions.

Supporting Information Available: Detailed experimental descriptions. This material is available free of charge via the Internet at http://pubs.acs.org.

IC048760O

---

(17) Preisler, R. S. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 609−616.
(18) Patel, D. J.; Kozlowski, S. A.; Nordheim, A.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 1413−1417.
(19) Keller, P. B.; Loprete, D. M.; Hartman, K. A. *J. Biomol. Struct. Dyn.* **1988**, *5*, 1221−1226.
(20) Song, B.; Sigel, H. *Inorg. Chem.* **1998**, *37*, 2066−2069.
(21) Smith, R. M.; Martell, A. E.; Chen, Y. *Pure Appl. Chem.* **1991**, *63*, 1015−1080.
(22) Fuertes, M. A.; Perez, J. M.; Gonzalez, V. M.; Alonso, C. *J. Biol. Inorg. Chem.* **1999**, *4*, 759−765.