Influence of Magnesium Ions on Spontaneous Opening of DNA Base Pairs

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Abstract

A large amount of experimental evidence is available for the effects of magnesium ions on the structure and the stability of the DNA double helix. Less is known, however, on how these ions affect the dynamics of the molecule and the stability of each individual base pair. The present work addresses these questions by a study of the DNA duplex [dCGCAGATCTGCG]$_2$, and its interactions with magnesium ions, using nuclear magnetic resonance (NMR) spectroscopy and proton exchange. Two-dimensional NMR experiments indicate that binding of magnesium to this DNA duplex does not affect its structure. However, even in the absence of structural changes, magnesium ions specifically affect the exchange properties of imino protons in the two GC/CG base pairs that are located in the interior of the double helix. These specific changes do not result from alterations in the rates of spontaneous opening of these base pairs. Instead, the changes most likely reflect an enhancement in the energetic propensity for spontaneous opening of the GC/CG base pairs that is induced by the binding of magnesium ions.

Keywords

proton exchange; base-pair opening; divalent metal ions; NMR spectroscopy; structural energetics

INTRODUCTION

Magnesium ions play key roles in the structure and the enzymology of DNA. As counterions, they interact with the phosphate groups, thus neutralizing the negative charges on the DNA backbone.[1] The ions can also bind to specific functional groups on DNA nucleobases, and this mode of binding is finely modulated by the DNA base sequence.[2-4] The sequence also determines, in large part, the changes in DNA structure resulting from binding of the ions. For example, it has been shown that Mg^{2+} induces the B-to-Z transition of poly(GC)[5] and the bending of the helix axis for DNA sequences containing tracts of AT base pairs.[6] Regardless of the base sequence, the binding of Mg^{2+} ions profoundly affects the physicochemical properties of the DNA. The largest effects are on the stability of the double helix. Early investigations have established that the melting temperature of the DNA increases with increasing Mg^{2+} concentration.[7] This stabilization has been attributed to the decrease in electrostatic repulsion between phosphate groups upon binding of the positively charged Mg^{2+} counterions.[8] Molecular dynamics simulations have augmented this picture by showing that the presence of Mg^{2+} also attenuates the fluctuations of the phosphate backbone and of the water molecules associated with it.[9] Furthermore, high-precision

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ultrasonic velocity measurements have demonstrated that binding of Mg^{2+} to DNA also results in a dehydration effect, which depends on the structure of the Mg^{2+}-DNA complex.[10]

The present work investigates the effects of Mg^{2+} ions upon another fundamental property of the DNA, namely, the spontaneous opening of individual base pairs. The opening of base pairs in DNA, at temperatures below the melting temperature, is a prerequisite for many key biological processes. Among these, replication of DNA into two daughter molecules and transcription of the DNA base sequence into messenger RNA both require the local opening of the double helix such that the base sequence of one strand can be used as a template. The repair of damage in DNA also relies upon opening of single base pairs: to be acted on, the damaged base must be displaced from the helical stack into a position where it is accessible to the repair machinery of the cell. The enzymes involved in these and many other processes use Mg^{2+} as a cofactor.[11] The catalytic role of the ion in many of these reactions is, in large part, understood. However, it is not yet known if the ion itself also affects the opening of base pairs that is required for these reactions. The present work addresses this question using nuclear magnetic resonance (NMR) spectroscopy and proton exchange. The DNA investigated (Figure 1) is a dodecamer, and its base sequence is palindromic. The DNA was chosen for investigation because the resolution of its imino proton NMR resonances, on which the investigation relies, is excellent. The processes of base-pair opening in this DNA, in the absence of Mg^{2+} ions, have been previously characterized by our laboratory.[12-14]

EXPERIMENTAL METHODS

DNA samples

The DNA oligonucleotide was synthesized on a DNA synthesizer (Applied Biosystems. Model 381A) using solid-support phosphoramidite chemistry. The crude DNA was purified by reverse-phase HPLC in formate buffer at pH 7 (with a gradient of 5 to 32% acetonitrile in 39 minutes). The counterions were replaced with Na^+ ions by repeated centrifugation (5 times) through Centricon YM-3 tubes (Amicon) using 0.5 M NaCl. The sample was then desalted by repeated centrifugation (8 times) with water. The DNA concentration in the NMR samples was 1.3 mM (duplex). The samples also contained 2 mM triethanolamine, which was used to determine the pH of the sample directly inside the NMR tube as we described.[15] In all experiments reported in the paper the pH of the DNA samples was 8.6±0.1.

NMR experiments

The NMR experiments were performed on a Varian INOVA 500 spectrometer operating at 11.75 T. One-dimensional NMR spectra for samples in H_2O were obtained using the Jump-and-Return pulse sequence.[16] The proton exchange rates were measured by transfer of magnetization from water. The water proton resonance was selectively inverted using a Gaussian 180° pulse (5.8 ms). This was followed by a variable delay in which the magnetization is transferred from water protons to DNA imino protons. In order to prevent the effects of radiation damping upon the recovery of water magnetization to equilibrium a weak gradient (0.21 gauss/cm) was applied during this exchange delay. At the end of the exchange delay, a second Gaussian pulse (2.9 ms) was applied to bring the water magnetization back to the z-axis. The observation was with the Jump-and-Return pulse sequence. Twenty-four values of the exchange delay in the range from 2 to 600 ms were used in each experiment. The exchange rates were calculated from the dependence of the intensity of the imino proton resonance of interest on the exchange delay as we have described.[17,18] Two-dimensional nuclear Overhauser effect spectroscopy (NOESY) spectra were obtained for a DNA sample in D_2O (1.3 mM DNA duplex) at a mixing time of 150 ms.

Determination of Base-Pair Opening Parameters

The opening of individual base pairs in DNA is generally characterized from the exchange of imino protons (Figure 1) with solvent protons.[18,19] In the native DNA double helix, the imino protons are not accessible to solvent due to their location in the center of the structure and to their participation in hydrogen bonds. For the exchange to occur the base pairs must open. In this opening reaction, the hydrogen bond holding the imino proton is transiently broken, and the proton is moved into a solvent accessible state where it can be transferred to acceptors present in solution.[20,21] The exchange rate observed experimentally depends upon the kinetic parameters of the opening reaction as:[20]

$$k_{ex} = \frac{k_{op} \cdot k_{ex,open}}{k_{cl} + k_{ex,open}}$$

(1)

where k_{op} and k_{cl} are the rates of opening and closing of the base pair, and $k_{ex,open}$ is the rate of exchange from the open state. To determine the opening and closing rates one varies the rate of exchange from the open state, $k_{ex,open}$, by adding to the DNA solution increasing concentrations of a proton acceptor. In the present work the proton acceptor used was Tris-base. Increasing concentrations of Tris-base were obtained by adding to the sample small aliquots of a stock Tris solution (1 M) at pH 8.6. The concentration of Tris-base was calculated from the total Tris concentration and the pK value of Tris. The latter was determined by NMR at 15°C, by monitoring the chemical shifts of selected Tris proton resonances as a function of pH. The following values were found: pK= 8.55±0.01 in the absence of Mg^{2+} and Na^+ ions, pK=8.40±0.02 in the presence of 200 mM Mg^{2+} ions, and pK=8.46±0.01 in the presence of 500 mM Na^+ ions.

The rate of exchange of the imino proton from the open state of the base pair depends on the concentration of Tris-base B as:

$$k_{ex,open} = k_0 + \alpha \cdot k_B \cdot [B]$$

(2)

where k_0 is the rate of exchange from the open state in the absence of Tris, k_B is the rate constant for transfer of the imino proton to Tris-base in isolated nucleotides, and α is a factor that accounts for any differences in the rate of proton transfer between isolated nucleotides and open DNA base pairs, e. g., restricted accessibility of the proton acceptor to the imino proton in the open base. Previous investigations have shown that, for Tris-base, α is close to 1.[21] The rate constant k_B depends on the pK values of the imino group (pK_{NH}) and of Tris (pK_B) as:[22]

$$k_B = k_{coll} \cdot \frac{1}{1 + 0.27 \cdot 10^{(pK_{NH} - pK_B + 0.8)}}$$

(3)

where k_{coll} is the diffusion-controlled rate of collision between the imino group (in the open state of the base pair) and the proton acceptor (e.g., $4.6 \cdot 10^8$ M^{-1} s^{-1} for Tris-base at 15°C). The rate constants calculated based on Eq. 3 for Tris in the absence of Mg^{2+} ions are: $9.82 \cdot 10^6$ M^{-1} s^{-1} for the thymine imino proton and $3.07 \cdot 10^7$ M^{-1} s^{-1} for the guanine imino proton, at 15°C.

The final equation for the exchange rate is obtained by inserting Eq. 2 into Eq. 1 (with α=1):

$$k_{ex} = \frac{k_{op} \cdot (k_0 + k_B \cdot [B])}{k_{cl} + k_0 + k_B \cdot [B]}$$

(4)

Two kinetic regimes can be distinguished depending on how the rate of exchange from the open state compares with the rate of closing of the base pair. The EX2 regime occurs when the concentration of proton acceptor is low such that $k_{ex,open} \ll k_{cl}$. In this case, the observed rate of exchange is proportional to the rate of exchange from the open state (Eq. 1), and thus to the concentration of proton acceptor B:

$$k_{ex} = \frac{k_{op}}{k_{cl}} \cdot k_{ex,open} = K_{op} \cdot (k_0 + k_B \cdot [B])$$

(5)

where $K_{op} = \dfrac{k_{op}}{k_{cl}}$ is the equilibrium constant for opening of the base that contains the imino proton. The other regime, called EX1 regime, occurs at high concentrations of proton acceptor, when $k_{ex,open} \gg k_{cl}$. In this regime, the exchange occurs in each opening event and

$$k_{ex} = k_{op}$$

(6)

RESULTS

The effects of Mg^{2+} ions upon the structure of the DNA dodecamer were investigated in two-dimensional NOESY experiments. Figure 2 illustrates the NOESY spectra in the absence and in the presence of Mg^{2+} ions. It is readily seen that the spectral positions of the cross-peaks in the two spectra are similar. The largest changes are observed for the terminal C_1 base, namely: upon adding 200 mM Mg^{2+}, C_1-H8 resonance shifts from 7.61 to 7.48 ppm, C_1-H2′ resonance shifts from 1.96 to 1.84 ppm, and C_1-H2″ resonance shifts from 2.37 to 2.29 ppm. A full comparison of the spectral positions of NOESY cross-peaks in the absence and in the presence of Mg^{2+} is shown in Figure 3. The figure contains 63 resolved cross-peaks corresponding to NOESY intra- and inter-nucleotide connectivities between H8, H6, H5, and CH_3 protons on the nucleobases, between nucleobase protons and deoxyribose H1′, H2′, H2″, H3′ and H4′ protons, and between deoxyribose H1′ and H2′/H2″ protons. The chemical shifts in the presence of 200 mM Mg^{2+} are within 0.15 ppm from those in the absence of Mg^{2+}. These results clearly indicate that Mg^{2+} ions do not induce significant changes in the structure of the DNA dodecamer.

To investigate the effects of Mg^{2+} ions on base-pair opening we used the NMR resonances of the imino protons (i.e., H1 in guanine and H3 in thymine, Figure 1). These resonances are shown in Figure 4. Since the base sequence of the DNA is palindromic six imino proton resonances are expected. However, the resonance from the terminal CG_1 base pairs is not observed because, at 15°C, it is broadened by fraying at the ends of the helix.

The exchange of imino protons in the dodecamer has been previously characterized by our laboratory, in the absence of Mg^{2+} ions.[12,14] A summary of our previous results at 15°C is given in Figure 5. For the imino protons in AT_6, CG_3 and GC_5 the dependence of the exchange rates on Tris-base concentration is that predicted by Eq. 4 and, at high concentrations of Tris-base, the exchange approaches the limiting EX1 regime. The opening rates and equilibrium constants, obtained from fits to Eq. 4, are shown in the figure for each

of these three base pairs. For the AT_4 base pair, the exchange rate of the imino proton at 0.2 M Tris-base (~ 80 s^{-1}) is the highest rate that can be measured accurately by NMR using transfer of magnetization from water.[18] Due to this technical limitation we were unable to measure the exchange rates of this proton at Tris-base concentrations exceeding 0.2 M. The exchange rates of the GC_2 imino proton at Tris-base concentrations higher than 0.1 M (*not shown*) are also increased beyond the range suitable for NMR measurement due to fraying at the ends of the helix.

To characterize the effects of Mg^{2+} ions upon the opening of individual base pairs in the DNA dodecamer we have selected for investigation the two regimes of imino proton exchange discussed in the Materials and Methods section. The rationale for this choice is two-fold. In the EX2 regime, the equilibrium constant for the opening reaction, K_{op}, can be directly obtained from the linear dependence of the exchange rate on proton acceptor concentration (Eq. 5). In the DNA dodecamer, the EX2 regime is observed at Tris-base concentrations less than 0.15 M (shown shaded in Figure 5 and expanded in Figure 6). The EX1 regime is of interest because the exchange rate in these conditions provides the rate of opening of the base pair (Eq. 6). This regime is reached asymptotically at high Tris-base concentrations. Since these high concentrations cannot be readily reached experimentally, we have chosen as an approximation of the EX1 regime the exchange rates at the highest Tris-base concentration investigated, namely, 1 M (shown shaded in Figure 5).

The effects of Mg^{2+} ions upon the exchange of imino protons in the EX2 regime are shown in Figure 6. For both, AT_4 and AT_6 base pairs the exchange rates in the presence of 200 mM Mg^{2+} ions decrease such that the slope of the linear dependence of the exchange rate on Tris-base concentration becomes smaller. In contrast, for the CG_3 and GC_5 base pairs, Mg^{2+} ions increase the exchange rates of imino protons. Furthermore, the shape of the exchange curve changes such that the dependence of the exchange rate on Tris-base concentration is no longer linear over the range of Tris-base concentrations up to 0.15 M. Instead, due to the increases in the exchange rates, the EX2 regime is now restricted to Tris-base concentrations less than ~ 0.01M.

The effects of Mg^{2+} ions upon the exchange of imino protons of CG_3, GC_5 and AT_6 at 1M Tris-base concentration (i.e., at or near the EX1 regime) are shown in Figure 7. The results clearly show that, for the CG_3 and GC_5 imino protons, the exchange rates do not change significantly upon increasing the concentration of Mg^{2+} ions up to 200 mM. A decrease in the exchange rate (~ 10 s^{-1}) is observed for the AT_6 imino proton.

In order to determine if the effects shown in Figures 6 and 7 are specific for Mg^{2+} ions we have also carried out a parallel investigation of the effects of Na^+ ions upon the imino proton exchange in the dodecamer. The concentration of Na^+ ions chosen for this investigation (i.e., 500 mM) provides an ionic strength that is comparable to that from 200 mM Mg^{2+}. The results show that Na^+ ions have no effect upon the exchange of CG_3 and GC_5 imino protons in the EX2 regime (Figure 6) or in the EX1 regime (Figure 7). For the AT_4 and AT_6 base pairs, the effects of Na^+ upon imino proton exchange in the EX2 regime are in the same direction as those of Mg^{2+} ions, but they are smaller. Similarly, in the EX1 regime, the effect of Na^+ upon the exchange of AT_6 imino proton parallels that of Mg^{2+} ions (Figure 7).

DISCUSSION

The changes in the exchange properties of imino protons that we observed in the presence of Mg^{2+} ions (Figure 6) can originate from an effect of Mg^{2+} ions on the stability of DNA base pairs. A change in base pair stability alters the equilibrium constant for opening of the base pair, K_{op} and thus, the exchange rate of the imino proton (Eqs. 4 and 5). To define the

magnitude of this effect the exchange rates were fitted to Eq. 5. The equilibrium constants were obtained from the slopes of these linear fits (with $k_B = 9.82 \cdot 10^6$ M^{-1} s^{-1} for thymine and $k_B = 3.07 \cdot 10^7$ M^{-1} s^{-1} for guanine). For the CG$_3$ and GC$_5$ base pairs in the presence of Mg^{2+} ions, the exchange rates were fitted to Eq. 4. The opening rates were assumed to be the same as those in the absence of Mg^{2+} ions. This assumption is justified by our observation that, in (or close to) the EX1 regime (Figure 7), the exchange rates of guanine imino protons are constant at increasing Mg^{2+} concentrations. Hence, it is likely that Mg^{2+} does not affect the opening rates of the two base pairs. The equilibrium constants were calculated as $K_{op} = k_{op}/k_{cl}$, using the closing rates obtained from the fits.

The results for the calculated opening equilibrium constants are summarized in Table 1. For the AT base pairs, the equilibrium constants in the presence of 200 mM Mg^{2+} ions (K_{op}^{Mg}) are smaller than those in the absence of the ions (K_{op}^0), namely, $K_{op}^{Mg}/K_{op}^0 \sim 0.6$. Decreases in the equilibrium constants are also observed in the presence of 500 mM Na$^+$ ions, namely, $K_{op}^{Na}/K_{op}^0 \sim 0.7 - 0.8$. Hence, the Mg^{2+}-induced stabilization of the AT base pairs is a non-specific effect due, in large part, to the higher ionic strength of the solution in the presence of the ions. For the GC/CG base pairs, the effects of Mg^{2+} are in opposite direction and are much larger than those for AT base pairs. For CG$_3$, the opening equilibrium constant in the presence of Mg^{2+} is ~3-fold larger than that in the absence of the ions. Similarly, for GC$_5$, the equilibrium constant in the presence of Mg^{2+} is ~8-fold larger than that in the absence of the ions. By comparison, Na$^+$ ions decrease the opening equilibrium constants of these two base pairs and the effect is much smaller. Therefore, for the CG$_3$ and GC$_5$ base pairs, the effects of Mg^{2+} are specific to these ions. An insight into the origin of these Mg^{2+}-induced changes in the opening equilibrium constants for CG$_3$ and GC$_5$ is provided by our results in the EX1 regime. As discussed above, the results show that Mg^{2+} does not affect the opening rates of the two base pairs. Hence, the increases in the equilibrium constants cannot originate from increases in the opening rates. Instead, it is likely that the opening equilibrium constants in the presence of Mg^{2+} are larger because the rates of closing of the two base pairs are smaller.

The rate of closing of a DNA base pair is related to the equilibrium constant for opening by the equation:

$$k_{cl} = \frac{k_{op}}{K_{op}}$$

(7)

In the absence of Mg^{2+} ions, the rates of closing for CG$_3$ and GC$_5$ are $2.2 \cdot 10^7$ and $1.7 \cdot 10^7$ s^{-1}, respectively (Figure 5). Our present results suggest that Mg^{2+} ions decrease the closing rate for CG$_3$ ~3-fold, to $6.6 \cdot 10^6$ s^{-1}. Similarly, the closing rate for GC$_5$ is decreased ~8-fold, to $2.2 \cdot 10$ s^{-1}. The mechanisms responsible for these Mg^{2+}-induced effects are not yet understood. Nevertheless, since the observed effects are specific for GC/CG base pairs, the mechanisms most likely involve the interaction of Mg^{2+} ions with guanine. In B-DNA, Mg^{2+} ions bind preferentially to guanines.[8,23] High-resolution crystallographic structures of DNA have shown that the hexahydrated Mg^{2+} ion is coordinated to guanines in the outer sphere mode, via water molecules in its primary hydration shell.[2,4,24] The bound ion is stabilized by an extensive network of hydrogen bonds from water molecules to the N7 and O6 atoms of guanine as well as to neighboring bases and phosphate groups.[2,4,24] The base-pair opening reaction clearly perturbs this network of hydrogen bonds. In the closing reaction, the correct network of hydrogen bonds at the bound ion must be restored.

Formation of these hydrogen bonds may require a longer time, thus explaining why, in the presence of the ions, the closing reaction is slower than that in the absence of the ions.

The changes in the exchange properties of imino protons that we observed may also result from a change in the rate constant k_B for the transfer of the imino proton to Tris-base, in the presence of Mg^{2+} ions (Eqs. 4 and 5). Quantum mechanical calculations suggest that direct, inner sphere coordination of Mg^{2+} ion to the guanine N7 should increase the acidity of the imino group.[25] In turn, such an increase in acidity would facilitate the exchange of the imino protons. To analyze this effect we have fitted the exchange rates for CG_3 and GC_5 in the presence of Mg^{2+} to Eq. 4, while keeping the opening and closing rates the same as those in the absence of Mg^{2+}. The k_B values obtained from the fits are: $(3.8\pm0.7)\cdot10^8\ M^{-1}s^{-1}$ for CG_3 and $(6\pm1)\cdot10^8\ M^{-1}s^{-1}$ for GC_5. These values are comparable to the rate of collision between the imino group and Tris-base, e. g., $4.6\cdot10^8\ M^{-1}s^{-1}$ in the absence of Mg^{2+} ions at 15°C.[22] Hence, to account for our results, the rate constants k_B should increase into the diffusion-controlled limit. Such large increases would require substantial decreases in the pK value of the guanine imino group (Eq. 3). This fact is illustrated in Figure 8. One can readily see that the rate constant k_B reaches, for example, 90% of the collision rate when the pK value of guanine decreases by more than 2 pH units, namely from 9.46 to 7.2. At present, no experimental evidence is available to support such changes in the guanine pK value upon Mg^{2+} binding. Furthermore, it is unlikely that the outer sphere coordination of Mg^{2+}, which is generally observed in B-DNA structures, could result in such a large change in the guanine pK. One should also note that this mechanism requires that the Mg^{2+} ions induce a change in the pK in the open state of the base pair, which is the state from which the exchange occurs. This is unlikely since, as discussed above, the base opening reaction is expected to weaken the binding of Mg^{2+} to the guanine. Taken together, these arguments thus suggest that the effects of Mg^{2+} upon the rate constant for proton transfer, k_B, are probably small, and can not account for the Mg^{2+}-induced changes in the exchange of guanine imino protons that we observed.

CONCLUSIONS

In the present work we have shown that Mg^{2+} ions specifically affect the exchange properties of the guanine imino protons in DNA. The effects are most likely due to a thermodynamic enhancement of the spontaneous opening of GC/CG base pairs upon binding of Mg^{2+}. This enhancement is not accompanied by an increase in the rates of base-pair opening. Instead, our results suggest that Mg^{2+} ions slow down the closing reactions, and thus increase the lifetimes of the GC/CG base pairs in the open, extra-helical state. These changes point to a possible role of Mg^{2+} ions in modulating the accessibility of individual guanine bases to enzymes that act upon the open states of the DNA double helix.

Acknowledgments

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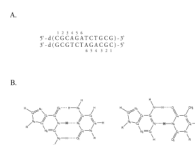(26). Ts'o, POP. Basic Principles in Nucleic Acid Chemistry. Vol. Vol. I. Academic Press; New York: 1974.

A.

5'-d(CGCAGATCTGCG)-3'
3'-d(GCGTCTAGACGC)-5'

B.

Figure 1.
A: The base sequence and numbering of base pairs in the DNA dodecamer investigated. **B**:
The structures of AT base pair (*right*) and GC base pair (*left*) with the imino protons
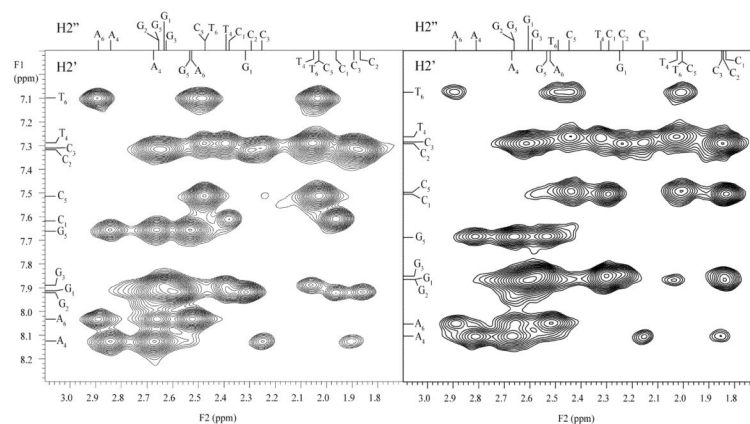highlighted.

Figure 2.
Selected region of the NOESY spectra of the DNA dodecamer in 50 mM Tris buffer in D_2O at pH 8.5 and at 15°C. The left spectrum is in the absence of Mg^{2+} and the right spectrum is in the presence of 200 mM Mg^{2+} ions. The cross-peaks are NOESY connectivities between H8/H6 protons on nucleobases (F1 dimension) and deoxyribose H2′/H2″ protons (F2 dimension).
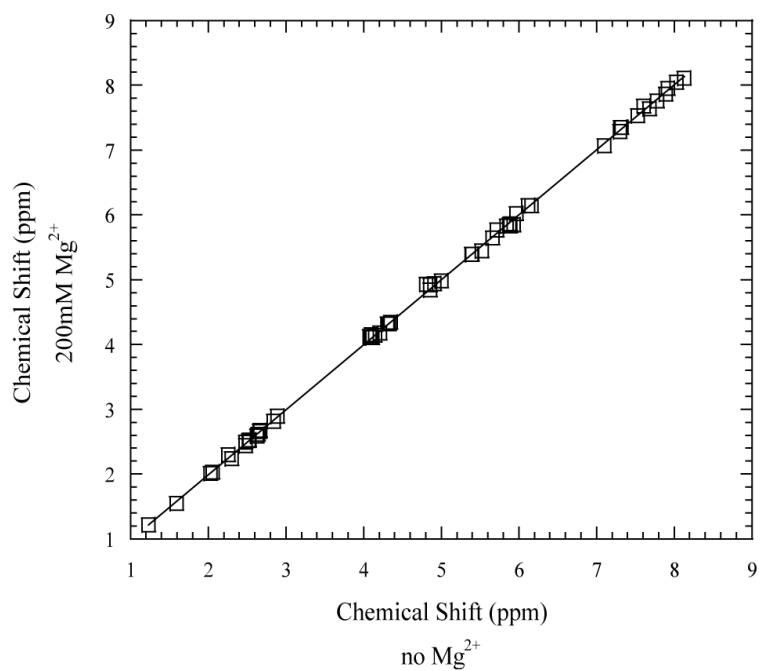
Figure 3.
Comparison of the chemical shifts of 63 NOESY cross peaks in the absence and in the presence of 200 mM Mg^{2+} ions. The data were fitted with a straight line with the slope 1.002±0.002.
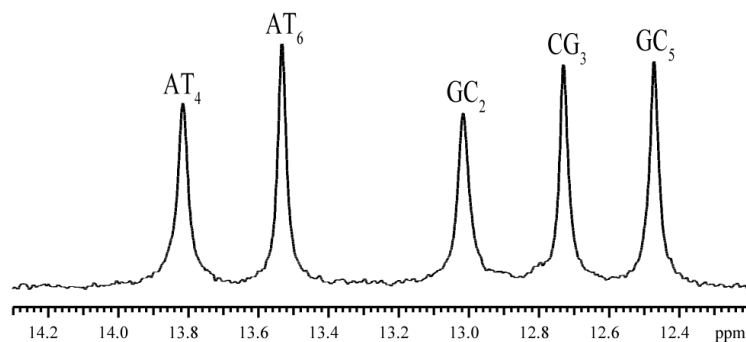
Figure 4.
The NMR resonances of the imino protons of the DNA dodecamer in 50 mM Tris with 2 mM triethanolamine, in 90%H_2O/10%D_2O at pH 8.6 and at 15°C. The assignments of the resonances to individual protons were previously obtained by this laboratory.[12]
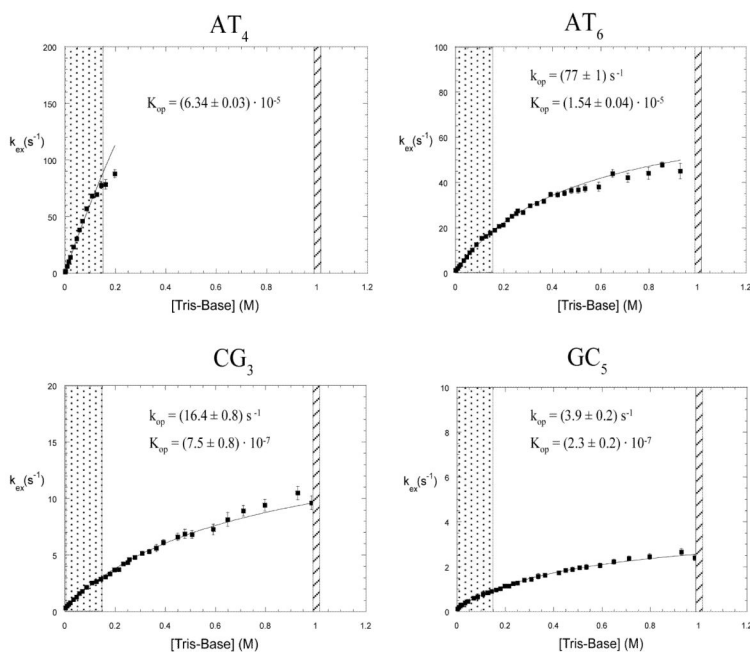
Figure 5.
Dependence of imino proton exchange rates on the concentration of Tris-base in the DNA dodecamer in the absence of Mg^{2+} ions, in $90\% H_2O/10\% D_2O$ at pH 8.6 and at 15°C.[14] The curves are non-linear least-squares fits to Eq. 4. The ranges of Tris-base concentrations investigated in the present work (EX2 and EX1 regimes, respectively) are shaded.
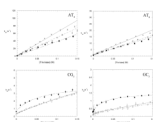
Figure 6.
Dependence of imino proton exchange rates on the concentration of Tris-base in the EX2 regime, in the absence of Mg^{2+} ions ($\times$), in the presence of 200 mM Mg^{2+} ions ($\blacksquare$) and in the presence of 500 mM Na^+ ions ($\square$). For CG_3 and GC_5 in the presence of 200 mM Mg^{2+} the curves are non-linear least-squares fits to Eq. 4. For all other exchange rates the lines are least-squares fits to Eq. 5.
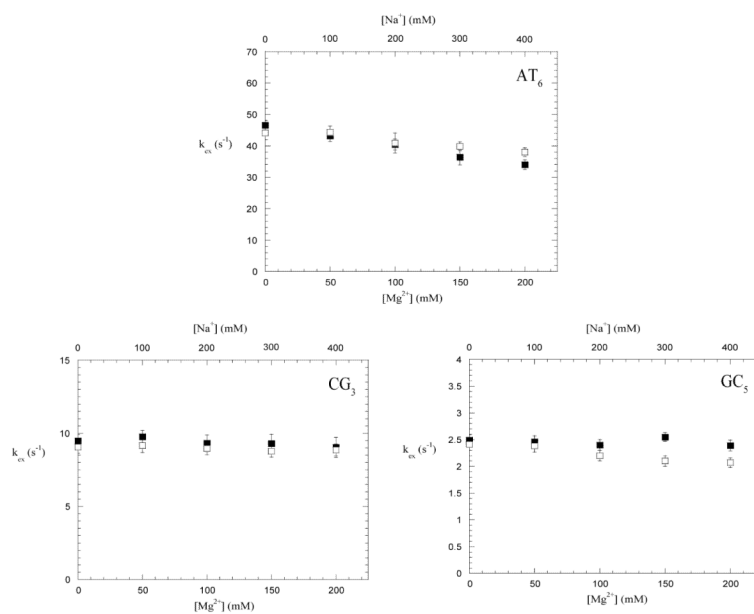
Figure 7.
Dependence of imino proton exchange rates on the concentration of Mg^{2+} ions (lower x-axis and ■) and Na^+ ions (upper x-axis and □) in the EX1 regime (i.e., 1M Tris-base concentration).
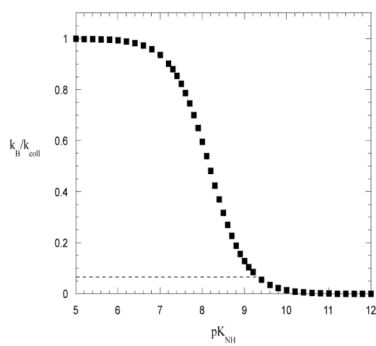
Figure 8.
Dependence of the ratio k_B/k_{coll} on the pK value of the imino group. The curve was obtained based on Eq. 3 using the pK value of Tris that we measured by NMR (pK = 8.40) and the pK value of the imino group in guanine (pK=9.46).[26] The horizontal line indicates the value of the k_B/k_{coll} ratio for the guanine imino proton in the absence of Mg^{2+} ions.

Table 1

Equilibrium constants for opening of individual base pairs in the DNA dodecamer in the absence of Na^+ and Mg^{2+} ions (K_{op}^0), in the presence of 200 mM Mg^{2+} ions (K_{op}^{Mg}), and in the presence of 500 mM Na^+ ions (K_{op}^{Na}).

Base Pair	K_{op}^0	K_{op}^{Mg}	K_{op}^{Na}
CG_3	$(7.5\pm0.8)\times10^{-7}$	$(2.5\pm0.5)\times10^{-6}$	$(5.9\pm0.1)\times10^{-7}$
AT_4	$(6.34\pm0.03)\times10^{-5}$	$(3.88\pm0.06)\times10^{-5}$	$(5.00\pm0.04)\times10^{-5}$
GC_5	$(2.3\pm0.2)\times10^{-7}$	$(1.8\pm0.3)\times10^{-6}$	$(1.49\pm0.07)\times10^{-7}$
AT_6	$(1.54\pm0.04)\times10^{-5}$	$(9.7\pm0.2)\times10^{-6}$	$(1.050\pm0.006)\times10^{-5}$