COUNTERION NMR IN POLYELECTROLYTE SOLUTIONS

$^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ interaction with DNA

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1. Introduction

The importance of electrostatic effects on the physico-chemical and biological properties of nucleic acids has long been recognised. It has been found that ion binding to nucleic acids cannot be described in a simple way in terms of equilibrium constants; therefore there has been a rapidly increasing interest in polyelectrolyte systems, concerning mainly the development of models amenable to theoretical analysis and the implications of polyelectrolyte phenomena in biological situations [1–5]. The concept of counterion condensation advocated by Manning (reviewed [1]) has been found to give a good first-order description of the interaction of ions with polyelectrolytes. According to this model, the condensation occurs above a certain critical linear charge density (1 elementary charge/7.1 Å for monovalent counterions and 1 charge/14.2 Å for divalent counterions), and in a solution containing only one type of counterion, the charge fraction of the polyelectrolyte remains constant over a broad concentration range.

For the study of counterion–polyion interactions at a molecular level, NMR should be a most useful method, and it has been shown, using $^{23}Na^{+}$ and $^{35}Cl^{-}$ as examples, that quadrupolar effects in NMR give significant information on the mode and dynamics of ion binding [6–9]. In particular the motional characteristics of Na^{+} bound to DNA and tRNA have been elucidated [9]. From a biological point of view, the study of Mg^{2+} and Ca^{2+} is especially intriguing. However, as discussed in [10], available isotopes have unfavourable NMR characteristics and Mg and Ca are therefore among the least studied elements. The use of isotope-enriched ^{25}Mg and ^{43}Ca considerably improves the situation; it was found that the interactions of Mg^{2+} [11] and Ca^{2+} [12] with a muscular protein, parvalbumin, produce marked effects in ^{25}Mg and ^{43}Ca quadrupole relaxation. However, one problem was encountered, that is, for high affinity binding to proteins, chemical exchange is often too slow to allow NMR studies.

Also $^{25}Mg^{2+}$ NMR has been used to study binding properties of bovine serum albumin and nitrogenase Fe protein [13]. The quadrupole relaxation for $^{25}Mg^{2+}$ has been shown sufficiently effective to give large line broadening effects also when the metal is bound to smaller ligands, such as small peptides, whereas for $^{43}Ca^{2+}$ the quadrupole relaxation in not so effective and in this case chemical shifts due to complexation could be observed [14,15].

It was anticipated in [12] that the Ca^{2+} and Mg^{2+} ion exchange should be faster when bound to biological polyelectrolytes than to specific sites in proteins, and as shown here, with DNA as an example, the metal ion binding may indeed be profitably explored.

2. Experimental

Calf thymus DNA of low molecular weight ($\sim 3.5 \times 10^5$; $S_{20} = 7.7$) was prepared as in [16]. The DNA-phosphate concentration was determined by the A_{260} assuming that 1.0 A_{260} is equivalent to 1.5×10^{-4} M DNA-P. ^{43}Ca and ^{25}Mg were purchased

from Oak Ridge, TN, as $^{43}CaCO_3$ (61% enrichment) and ^{25}MgO (98% enrichment), respectively. The ^{25}Mg and ^{43}Ca linewidth studies were made at 6.095 MHz and 6.73 MHz, respectively, on a modified Varian XL-100-15 spectrometer operating in the FT mode. External proton lock was employed, the acquisition times were 0.1 s for ^{25}Mg and 0.2 s for ^{43}Ca. The number of transients varied from 500–10 000, depending on linewidth and solution. Each reported linewidth is the average of at least 2 different measurements and the resulting errors are $< 5\%$. Temperature was maintained accurate to 0.5°C by a stream of dry thermostatted gas.

3. Results and discussion

In fig.1,2 we report the temperature dependence of $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ relaxation, respectively, in the presence of DNA. (In the absence of DNA, relaxation is much slower and decreases monotonically with increasing temperature, cf. [11].) The experimental

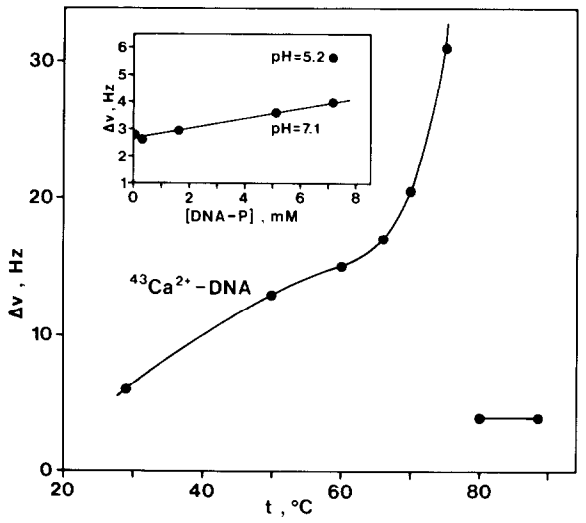

Fig.1. Study of the Ca^{2+}–DNA system by ^{43}Ca NMR. The dependence of the linewidth on temperature for a solution containing 7.1 mM DNA-P, 35 mM Ca^{2+}, 57 mM Na^+ in 13 mM Tris–HCl, at pH 5.2. Insert: Effect of DNA concentration on the ^{43}Ca linewidth. Successive volumes of a stock DNA solution (4.25×10^{-2} M DNA-P in 75 mM Tris–HCl, at pH 7.1) added to a 1.53 ml solution containing 40 mM Ca^{2+}, 65 mM Na^+ in 15 mM Tris–HCl, at pH 7.1. Temperature 27°C. Total volume variation 13%.

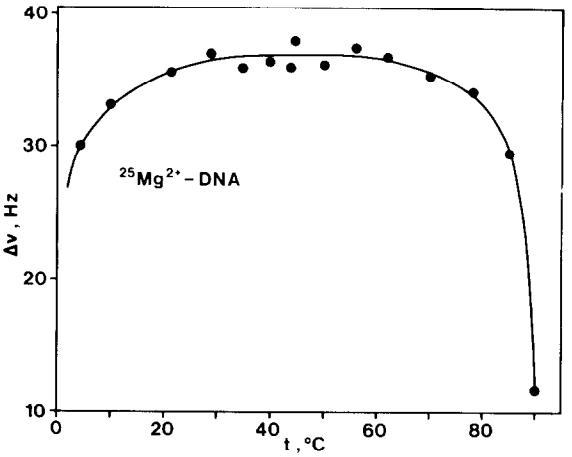

Fig.2. Dependence of the ^{25}Mg linewidth on temperature for a solution containing 7.1 mM DNA-P, 88 mM Mg^{2+}, 21 mM Na^+ in 50 mM Tris–HCl, at pH 7.4.

results are given as linewidths which are related to the transverse relaxation time, T_2, as $T_2^{-1} = \pi\Delta\nu_{\frac{1}{2}}$. The important features of fig.1 are the marked increase in relaxation rate with increasing temperature, and the low relaxation rate above the DNA melting point: this is considered to correspond to a transition from a double-stranded helical DNA to a single-stranded random coil state. For $^{25}Mg^{2+}$ (fig.2) there is likewise a marked decrease in relaxation at DNA melting while below this temperature there is a slow variation with a wide maximum. A similar curve is obtained for transfer RNA [17]. Parallel optical studies of the denaturation of double-stranded DNA in the presence of Ca^{2+} and Mg^{2+} show that the melting transition occurs at 80–85°C under conditions similar to those used for the NMR experiments.

The principles of ion quadrupole relaxation studies of macromolecular systems have been reviewed [18,19]. (In [18] a detailed account of the theory is given.) For both ^{25}Mg and ^{43}Ca, quadrupole interactions give the dominating relaxation mechanism. The observed relaxation can be written as a sum of contributions from different sites. That is:

$$\pi\Delta\nu_{\frac{1}{2}} = T_2^{-1} = \Sigma p_i/(T_{2i} + \tau_{ex,i})$$

Here p_i is the fraction of ions in site i, $\tau_{ex,i}$ is the life-time of the ion in site i and T_{2i} is the intrinsic

relaxation time of site i. $T_{2i}^{-1} = K\chi^2\tau_c$ where $K = 0.947$ for ^{25}Mg (spin 5/2) and 0.403 for ^{43}Ca (spin 7/2), χ is the quadrupole coupling constant describing the strength of the interaction between the ion and the site and τ_c the correlation time. Here we have assumed that the extreme-narrowing condition applies, i.e., $\omega\tau_c << 1$ (ω is the resonance Larmor frequency in rad/s); this is not established for these ions but does not affect the general conclusions of this work.

Current polyelectrolyte theories [1–5] relate counterion binding to the charge density of the cylinder or to a linear charge density; the Manning ion condensation model [1] is in qualitative agreement with solutions of the Poisson-Boltzmann equation for different geometries [20]. The linear charge density is extensively decreased at the melting of DNA, and the dramatic counterion relaxation changes at high temperatures are in qualitative agreement with the transition from a double- to a single-stranded DNA. According to the Manning ion condensation model [1], considering only the linear charge density, the Mg^{2+} and Ca^{2+} binding should change from ~88% polyion charge neutralization to a much lower level (~50%). From the temperature dependence of counterion relaxation it can be deduced, at least for Ca^{2+}, that $\tau_{ex,i}$ dominates over T_{2i} [18]. With the aid of the Manning condensation model (88% charge neutralisation) and the measured line broadenings, the average lifetime of Mg^{2+} bound to DNA was estimated to be ~0.4 ms at 4°C while Ca^{2+} has an average lifetime of ~9 ms at 25°C and ~3 ms at 50°C. It is clear that analysis of the data of fig.1,2 in terms of activation parameters must be preceded by rather detailed studies of the binding equilibria.

If the ion condensation model were applicable under the conditions of this work, one would expect that the difference between the observed linewidth, and that of a solution without DNA, should be proportional to the DNA concentration at a constant ion concentration. As shown in fig.3, there is a marked non-linearity in the plot of linewidth of ^{25}Mg versus DNA concentration which is in contrast to many other macromolecular systems [18]. It has also been observed that the excess linewidth of $^{23}Na^+$ in transfer RNA solutions is not linear with respect to tRNA concentration [17]. However, it is generally not expected that the charge fraction of a polyelectrolyte is independent of concentration.

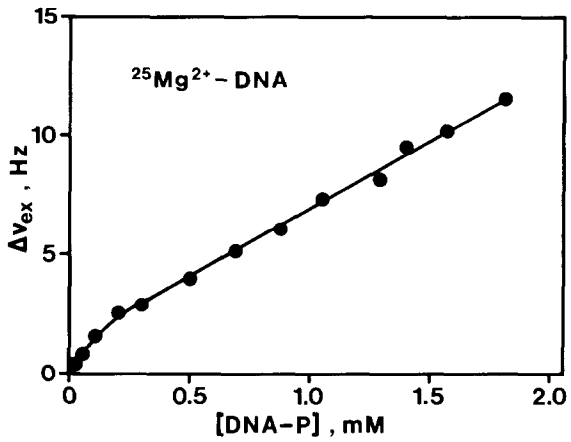

Fig.3. Dependence of the ^{25}Mg excess linewidth on DNA concentration at 28°C. Successive volumes of a stock DNA solution (1.54×10^{-2} M in 75 mM Tris–HCl, at 7.1) added to a 1.50 ml solution containing 96 mM Mg^{2+}, 17 mM Na^+ in 15 mM Tris–HCl, at pH 7.1. Total volume variation 13%.

This report clearly demonstrates the feasibility of NMR studies of Ca^{2+} and Mg^{2+} interaction with DNA and also shows that direct access to both kinetics and thermodynamics of ion binding can be obtained. The kinetic information is particularly difficult to obtain by other techniques, and the marked difference in exchange kinetics between Mg^{2+} and Ca^{2+}, which probably reflects a difference in hydration state of the bound ions, will be examined in detail elsewhere.

Acknowledgements

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