^{23}Na NMR AS A PROBE OF ION BINDING TO CHROMATIN

Dennis R. BURTON and Pétur REIMARSSON
Physical Chemistry 2, Chemical Centre, Lund Institute of Technology, POB 740, S-220 07 Lund, Sweden

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1. Introduction

The ionic interaction between the negatively-charged phosphate groups of DNA and the positively-charged basic amino acid residues of the histones is of prime importance in maintaining the structural integrity of the chromatin complex. However there is a far from exact mutual neutralisation between these two charged species in chromatin [1,2], e.g., it has been estimated that only approx. 60% DNA phosphates are neutralised by histone basic residues, the rest being taken up by Na^+ ions [1]. In an exploratory study we have investigated the interaction of the Na^+ ion with a calf thymus chromatin preparation using ^{23}Na NMR. Studies of this quadrupolar nucleus can yield binding and dynamic information in macromolecular systems (reviewed [3]). Specifically we have studied ^{23}Na relaxation behaviour for chromatin solutions with increasing salt concentration corresponding to an increased dissociation of the histone from the DNA. The results allow us to compare the binding of Na^+ in DNA and in the chromatin complex. Conclusions can then be drawn about the possible involvement of this ion in specific interactions.

2. Experimental

Chromatin was prepared as in [4] with the exception that after the last washing portions of the chromatin pellet were taken up directly in an NaCl solution buffered with 10 mM phosphate to pH ~6.2.

DNA concentration was measured as A_{260} using an extinction coefficient of 6600/mol phosphorus.

^{23}Na relaxation times were measured at 23.81 MHz on a Bruker BKr-322s pulsed spectrometer interfaced with a Varian V-71 computer. T_1 was measured using a $180°$–τ–$90°$ pulse sequence and T_2 by means of the Meiboom-Gill modification of the Carr-Purcell sequence. A signal to noise ratio of at least 50:1 for each measurement was obtained by time averaging. Each T_1 value was the average of at least 3 independent measurements (12 integrations) and each T_2 the average of at least 8 measurements. Chromatin/NaCl and the corresponding NaCl solutions were measured consecutively to minimise errors. The probe temperature was maintained at 4±0.5°C by a stream of dry thermostatted gas.

Linear dichroism (LD) measurements were performed at 260 nm as in [5,6].

3. Theory

For the spin 3/2 nucleus ^{23}Na in a NaCl/macromolecule solution, where the Na^+ ion interacts with the macromolecule, the longitudinal and transverse magnetisations generally decay non-exponentially. However for cases where $\omega^2\tau_c^2$ (see below) is of the order of unity the decays are approximately exponential. In this case the differences between the rates in the presence (T_i^{-1}) and absence (T_{i0}^{-1}) of macromolecule can be written approximatively as [7]:

$$(T_1^{-1})_{ex} = T_1^{-1} - T_{10}^{-1} = \sum_i \frac{2\pi^2}{5} p_i \nu_{Qi}^2 \tau_{ci} \left(\frac{0.8}{1+4\omega^2\tau_{ci}^2} + \frac{0.2}{1+\omega^2\tau_{ci}^2}\right) \quad (1)$$

$$(T_2^{-1})_{ex} = T_2^{-1} - T_{20}^{-1}$$

$$= \sum_i \frac{\pi^2}{5} p_i \nu_{Qi}^2 \tau_{ci} \left(0.6 + \frac{0.6}{1+\omega^2\tau_{ci}^2} + \frac{0.4}{1+4\omega^2\tau_{ci}^2} + \frac{0.4}{1+\omega^2\tau_{ci}^2}\right) \quad (2)$$

when fast exchange conditions apply as was verified in our case by temperature dependence studies. In these equations p_i is the probability of an ion being found at site i on a macromolecule, ν_Q is the quadrupole coupling constant, τ_c is the correlation time characterising the time dependence of the field gradients and ω is the Larmor frequency.

The dissociation of histones from DNA in increasing salt concentration can be envisaged to affect the excess ^{23}Na relaxation rates in principle in two ways.

In the first instance effects acting equally on $(T_1^{-1})_{ex}$ and $(T_2^{-1})_{ex}$ and therefore leaving T_1/T_2 unchanged can be considered. Thus an increase in salt concentration will in itself lead to a decrease in p_i and therefore in $(T_i^{-1})_{ex}$. The effect of increased dissociation with increasing salt concentration will however tend to increase p_i. The net effect in $(T_i^{-1})_{ex}$ may be hard to predict.

The second type of effect is one in which changes in T_1/T_2 do occur. Such changes should reflect the creation or loss of ion binding sites characterised by different τ_c values.

4. Results and discussion

4.1. *^{23}Na relaxation*

Figure 1 shows the excess ^{23}Na relaxation rates, normalised to the same chromatin concentration, for a chromatin/salt solution as the concentration of salt is increased. The chromatin concentration employed approached the lowest at which significant effects on ^{23}Na relaxation could be observed. From fig.1 no significant effect in $(T_1^{-1} - T_{10}^{-1})$ can be seen. However there is a marked decrease in $(T_2^{-1} - T_{20}^{-1})$ and it would appear that the ratio T_1/T_2 decreases as the salt concentration is increased. This is seen more clearly in fig.2 where a higher concentration of chromatin was used so that the excess relaxation rates were greater and the effects of experimental

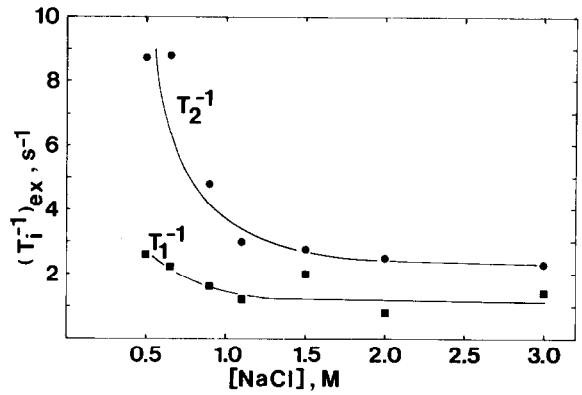

Fig.1. The effect of increasing NaCl concentration on ^{23}Na excess relaxation rates in the chromatin/NaCl system. The DNA concentration in each case was within 20% of 4.3 mM in phosphate (A_{260} = 28.7) and relaxation rates are normalised to this concentration: (■) $(T_1^{-1})_{ex}$; (●) $(T_2^{-1})_{ex}$.

error correspondingly less. Thus from fig.1,2 it can be concluded that at low salt (0.5 M) the ratio T_1/T_2 is approx. 3 and this decreases to approx. 1–1.6 at high salt concentration.

The reason for these observations must lie in differences in the magnitude of the correlation time τ_c

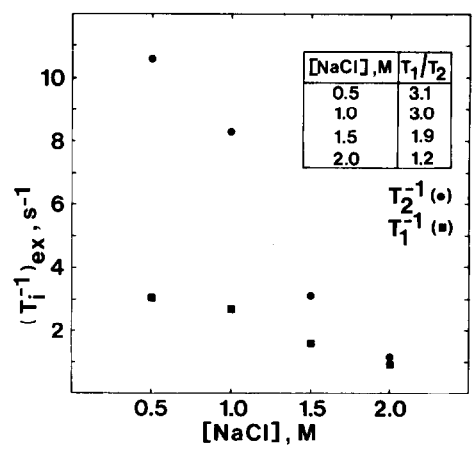

Fig.2. The effect of increasing NaCl concentration on ^{23}Na excess relaxation rates in the chromatin/NaCl system at a higher DNA concentration. The relaxation rates are normalised to a DNA concentration of 5.6 mM in phosphate (A_{260} = 37). Results obtained with a different chromatin preparation to that in fig.1: (■) $(T_1^{-1})_{ex}$; (●) $(T_2^{-1})_{ex}$.

in the low and high salt cases. If ω is the resonance frequency then $\omega^2\tau_c^2>1$ for the low salt case (so-called non-extreme narrowing conditions) whereas $\omega^2\tau_c^2\sim1$ for the high salt case (where the condition $\omega^2\tau_c^2 \ll 1$ corresponds to extreme narrowing and $T_1/T_2 = 1$ (see eq. (1) and eq. (2)). τ_c is hence longer for the low than the high salt case.

The question then arises as to the species present at the different salt concentrations. Studies [8] have indicated that at 0.5 M NaCl only histone H1 has been dissociated from the chromatin complex leaving the tertiary structure essentially intact whereas complete dissociation of the complex into DNA and histones has occurred at roughly 1.5–2 M NaCl. These figures of course depend on chromatin concentration but provide a rough guide. We have further checked 'nativity' of chromatin structure by linear dichroism (LD) as described below. The ^{23}Na relaxation behaviour of calf thymus DNA was also checked at low salt concentration. No significant difference in the ratio T_1/T_2 was observed between low and high salt concentrations.

The ^{23}Na relaxation measurements thus indicate the existence of Na^+ binding sites in chromatin of a different nature to those found in DNA.

Further support for this viewpoint comes from the results presented in table 1 where the effect of urea on ^{23}Na relaxation at low salt concentration is presented. As the urea concentration is increased and the chromatin structure denaturated there is a marked decrease in the T_1/T_2 ratio.

Our main conclusion is independent of the nature of τ_c. However, it is of interest to consider its possible nature. τ_c is defined by the equation:

$$\tau_c^{-1} = \tau_R^{-1} + \tau_M^{-1}$$

Table 1
The effect of urea on ^{23}Na excess relaxation rates in a solution of chromatin in 0.65 M NaCl

	$(T_1^{-1})_{ex}$	$(T_2^{-1})_{ex}$	T_1/T_2
Chromatin	4.7	16.9	3.6
Chromatin + 6 M urea	4.9	12.3	2.5
Chromatin + 8 M urea	6.1	12.9	2.1

The DNA concentration was 9.1 mM in phosphate (A_{260} = 60)

where τ_R is a rotational correlation time and τ_M the lifetime of the ion in the bound state. ^{23}Na NMR [9] has equated τ_c for the Na^+–DNA interaction, found to be approx. 5 ns, with τ_R. The longer τ_c for the Na^+–chromatin interaction in this case would reflect Na^+ sites involving restricted motional freedom. However the observation that the lifetime, τ_M, of the divalent cation, Co^{2+}, on DNA has an upper limit of 10^{-5} s [10] raised the possibility that for the monovalent cation, Na^+, $\tau_c=\tau_M$. In this latter case our results would reflect a decreased access of Na^+ ions to specific sites on chromatin.

Finally two further considerations with respect to the ^{23}Na relaxation data are in order. No evidence of non-exponentiality was observed in the decays of either the longitudinal or transverse magnetisations of ^{23}Na indicating the validity of the approximate equations (1) and (2). We did not either observe any significant difference in intensity of the integrated ^{23}Na resonance between NaCl and chromatin/NaCl solutions. Second the non-equality of T_1 and T_2 is indicative of fast exchange conditions as would be expected. This was verified by temperature dependence studies when the relaxation rates $(T_i^{-1}-T_{i0}^{-1})$ i=1,2 were found to decrease with increasing temperature.

4.2. *Linear dichroism (LD)*

The LD for native chromatin (chromatin in 0.7 mM phosphate buffer) was found to be an order of magnitude less than that for DNA (chromatin in 2 M NaCl). The LD for chromatin in 0.5 M NaCl was close to that for native chromatin suggesting the structure to be essentially intact at this low salt concentration. Most of the change in LD occurred between 0.5 M and 1.5 M NaCl.

5. Conclusion

We stress that the results described here pertain to a chromatin preparation less well-defined than that obtained by enzymatic digestion. Nevertheless we feel that we have demonstrated the potential of ^{23}Na NMR relaxation measurements in the study of the role of monovalent cations in the chromatin structure. It seems very likely that application of the method to better-defined particles, e.g., nucleosomes will allow

us to make more quantitative conclusions in the future.

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