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The interaction of polyamines with DNA: a ^{23}Na NMR study

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ABSTRACT

The interaction between a variety of polyamines, both naturally occurring and synthetic, and calf thymus DNA has been studied using ^{23}Na NMR. The relaxation behaviour of ^{23}Na reflects the extent of interaction of Na^+ with DNA phosphate groups and therefore the extent of charge neutralisation of DNA phosphate groups (P) by polyamine amino and imino groups (N) in solutions of DNA, polyamine and Na^+. The studies reveal that whereas spermine and spermidine are capable of expelling nearly all of the Na^+ ions from DNA at $N/P \sim 1$, diamines such as putrescine and homologues of spermine and spermidine are capable of neutralising only roughly 50% of DNA phosphates. The results provide a challenge to current models of DNA-polyamine interactions.

INTRODUCTION

The interaction of naturally occurring polyamines like putrescine, spermine and spermidine with DNA and RNA has long been recognized[1,2]. Polyamines have a profound effect on protein synthesis and other cellular events[1]. Spermine and spermidine are observed not only in most cells but also in certain bacteriophages[3,4] and it has been suggested that polyamine-DNA interactions are responsible for viral DNA compaction *in vivo*[5]. In an *in vitro* electron microscopic study[6] it has indeed been observed that T7 DNA may be brought to a collapsed form (spheroids or "donuts") by the addition of spermine and spermidine. Similar structures have been observed in electron micrographs of ruptured T7 phages[7] as well as of other phages and animal viruses[8,9].

Spermidine and putrescine are required for the optimal rate of protein synthesis of *E. Coli* ribosomes in an *in vitro* system[10] - a finding that may be related to the well known effects of polyamines on tRNA folding[11,12]. The determination of the crystal structure of yeast $tRNA^{Phe}$ to a high resolution was made possible by the use of spermine additions[13,15].

Several models for the structure of polyamine - DNA complexes have been proposed. As early as 1964 Tsuboi suggested that the spermine molecule (NH_2-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3NH_2$) interacted with the B-form of DNA in such a way that the central tetramethylene chain spans across the major groove allowing close contact between two neighbouring phosphate groups on each strand and the two amine groups on each half of the polyamine molecule[16]. In later models by Liquori et al.[17], Suwalsky et al.[18] and Tsuboi[19], spermine is assumed to bridge across the minor groove rather than the major groove. The energy of interaction of a spermine molecule with the A and B forms of DNA has recently been calculated assuming the polyamine to be located in the minor groove[20]. Somewhat larger interaction energy is calculated for the A form than for the B form. The location of spermine in tRNA crystals has been reported[15,21]. One spermine molecule is found in the deep groove of the double helix of the anticodon stem with its amino and imino groups in close proximity to phosphate groups. To what extent this observation can be carried over to DNA-polyamine interactions is presently not clear.

Most models of polyamine-DNA complexes depend in one way or another on the assumption of an energetically favourable match between the amino and imino groups of the polyamine molecule and the phosphate groups of the DNA duplex. The occurrence or non-occurrence of certain types of polyamines in Nature should according to this hypothesis reflect their geometrical fit with the DNA molecule. In the present work we have attempted to test this idea by studying the extent of charge neutralization of the phosphate groups of DNA that can be accomplished by the addition of various structurally different polyamines. The degree of charge neutralization of the DNA molecule has been monitored using ^{23}Na NMR to probe the interaction of sodium ions with the polyanion. NMR studies of ion binding to macromolecules is an established method for studies of binding properties on a molecular level [22,25] and NMR studies of alkali ions have previously been employed to investigate various aspects of cation-DNA interactions[26,29].

The ^{23}Na NMR data reported here indicate the degree of charge neutralization of DNA accomplished by different polyamines to be strongly dependent upon the molecular structure of the amine in a manner not anticipated.

MATERIALS AND METHODS

Calf thymus DNA (highly polymerised) and high grade quality polyamines were purchased from Sigma (Poole, England) except 1,2 bis-(3-aminopropyl) ethylenediamine purchased from Aldrich (Karlsruhe, BRD). The DNA was dissolved in 30 mM sodium phosphate buffer at pH 6.8 and procedures liable to fracture the DNA such as pipetting avoided. DNA concentration was measured as A_{260} using an extinction coefficient of 6600/mol phosphorus. Polyamine stock solutions of ~100 mM were prepared in the same phosphate buffer as above.

^{23}Na NMR measurements were performed at 26.45 MHz on a modified Varian XL-100/15 FT NMR spectrometer. The line-shape at this frequency was found to be lorentzian for all cases studied and the line-width measured at half-height ($\nu_{\frac{1}{2}}$). Sample volumes used were 3 ml and the temperature generally 4 $\pm$ 1°C. A number of measurements at 23°C showed the general effects to be described were also found at the higher temperature.

Under the conditions of our experiments the polyamines are expected to be fully positively charged. For putrescine (diaminobutane) this was confirmed by ^{13}C NMR when the pK_a's of the NH_2 groups were found to be ~10.2.

THEORY

^{23}Na is a magnetic nucleus with spin quantum number I = 3/2. The nucleus has an electric quadrupole moment with a value of about $0.11 \times 10^{-28} m^2$ and its relaxation is mainly due to interactions between the electric quadrupole and time-varying electric field gradients at the nucleus[30]. The magnitude of the coupling between the nucleus and the field gradients is usually expressed by a nuclear quadrupole coupling constant (X). The time variation of the field gradients depends on the motional properties of Na^+ and is expressed by a correlation time (τ_c). Finally, ^{23}Na bound to DNA is not observed directly - under the conditions of our experiment an averaging between free and bound sites generally takes place ("fast chemical exchange", cf. also ref. 26-29). Under such conditions the observed ^{23}Na transverse relaxation rate R may be written

$$R = p_F R_F + p_B R_B \qquad (1)$$

where p_F and p_B are the mole fractions of free and bound $^{23}Na^+$; R_F is the relaxation rate of "free" $^{23}Na^+$ ions, i.e. ions far enough from

the polyanion to be in essence unaffected by its electric field, and R_B is the mean relaxation rate of all $^{23}Na^+$ ions associated with the DNA molecule. R_F for $^{23}Na^+$ in moderately concentrated solutions is of the order of 16-17 sec^{-1} [31] and the value of R_B in the presence of DNA is about 200 sec^{-1} [27-29]. Rearranging (1) with $p_B R_B = R_{ex}$, the excess relaxation rate in the presence of DNA, then:

$$R_{ex} = R - p_F R_F \approx R - R_F \qquad (2)$$

if $p_F \sim 1$. R_{ex} (or the excess linewidth $\Delta\nu_{ex} = R_{ex}/\pi$) is therefore measured as the difference in ^{23}Na relaxation rate in the presence and absence of DNA. Species competing with Na^+ in binding to DNA will reduce p_B and hence R_{ex}. This is the basis of the use of ^{23}Na NMR to monitor polyamine binding to DNA.

The interpretation of the polyamine competition experiments reported here do not depend on the detailed molecular mechanism of ^{23}Na relaxation at the polyelectrolyte DNA - a problem that has not yet been satisfactory solved[24,25]. The interpretation is, however, dependent on the approximate constancy of the term R_B during the competition experiments. Previous ^{23}Na NMR studies on DNA solutions indicate that the value of R_B is essentially independent of salt concentration and the simultaneous presence of other monovalent and divalent cations[28,29,32]. Furthermore, ^{23}Na relaxation measurements at high magnetic field (6.0 T) using methodology described elsewhere[22,24,25,32,33] reveal that changes in τ_c on titration of DNA with putrescine are small making very little contribution to the overall observed changes in ^{23}Na relaxation rates. Nevertheless, in using ^{23}Na NMR relaxation as a titration indicator of polyamine binding to DNA, the possibility of changes in R_B should be borne in mind when considering the finer details of ^{23}Na relaxation behaviour.

RESULTS AND DISCUSSION

Figures 1-3 show the excess ^{23}Na linewidths, $\Delta\nu_{ex}$, ($\Delta\nu_{ex} = R_{ex}/\pi$) in solutions of calf thymus DNA in the presence of different types of polyamines. The excess linewidths are plotted as a function of the number of positive amino or imino groups per phosphate group on DNA (N/P). The detailed conditions of the experiments are given in the legends to the figures.

The polyamines have been grouped according to their total charge. Figure 1 illustrates the effect of titrating the DNA solution

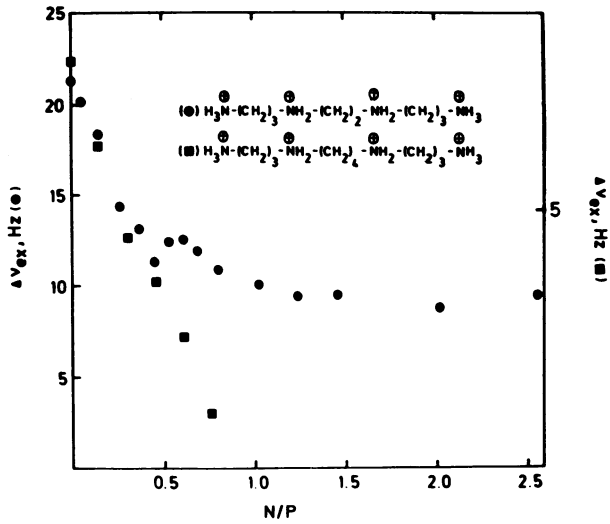

Fig. 1 - The excess ^{23}Na linewidth ($\Delta\nu_{ex}$) as a function of added tetraamine in a solution of calf thymus DNA, polyamine and sodium phosphate.

N/P refers to the ratio of polyamine nitrogen to DNA phosphate. (●), 1,2 bis(3-aminopropyl) ethylene diamine. DNA concentration was 12.6 mM in phosphate; buffer was 30 mM sodium phosphate (57.6 mM total Na^+). (■), spermine. DNA was 4.0 mM in phosphate, buffer was 30 mM Na phosphate (49.0 mM total Na^+). Note the different scale for spermine from all other polyamine results reported here. A lower concentration of DNA than normal was used with spermine owing to precipitation problems at higher DNA concentrations. For the conditions used DNA precipitated irreversibly at N/P$\sim$ 1.

with the tetraamines spermine ($\overset{+}{N}H_3 - (CH_2)_3 - \overset{+}{N}H_2 - (CH_2)_4 - \overset{+}{N}H_2 - (CH_2)_3 \overset{+}{N}H_3$; N-3-N-4-N-3-N) and 1,2 bis (3-aminopropyl) ethylenediamine ($\overset{+}{N}H_3-(CH_2)_3-\overset{+}{N}H_2-(CH_2)_2-\overset{+}{N}H_2-(CH_2)_3-\overset{+}{N}H_3$; N-3-N-2-N-3-N). Figure 2 compares titrations for the triamines spermidine ($\overset{+}{N}H_3-(CH_2)_3-\overset{+}{N}H_2-(CH_2)_4-\overset{+}{N}H_3$; N-3-N-4-N) and bis (3-aminopropyl)amine (homospermidine, $\overset{+}{N}H_3-(CH_2)_3-\overset{+}{N}H_2 -(CH_2)_3-\overset{+}{N}H_3$; N-3-N-3-N). Figure 3, finally, illustrates the titrations with the diamines, 1,2-diaminoethane (N-2-N) 1,3-diaminopropane (N-3-N) and 1,4-diaminobutane (putrescine; N-4-N). For putrescine two curves obtained under different conditions are shown.

If the structural details of the polyamines studied here could be neglected and the amines were behaving as pseudo-ions with total charges, Z, equal to 2, 3 and 4, one would expect their ability

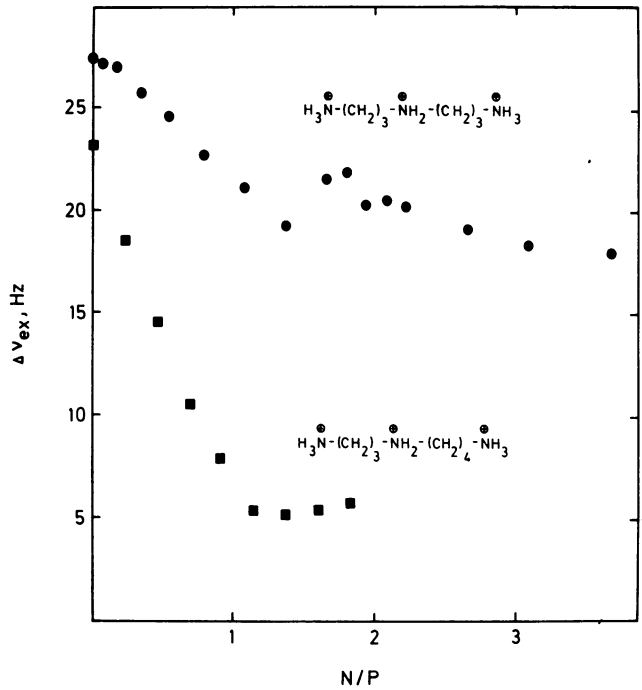

Fig. 2 - The excess ^{23}Na linewidth as a function of added triamine in a solution of DNA, polyamine and sodium phosphate.
(●), homospermidine. DNA was 16.0 mM in phosphate, buffer was 30 mM Na phosphate. (■), spermidine. DNA was 15.8 mM, buffer was 30 mM Na phosphate. Precipitation occurred with spermidine at N/P ~ 2.

to compete with sodium ions associated with DNA to be (a) the same for all polyamines with the same Z and (b) to increase with increasing Z. The experimental data are clearly at variance with this simple model. There are large differences between polyamines of equal Z and some diamines are just as effective competitors for DNA-bound sodium as is the tetraamine N-3-N-2-N-3-N or even more efficient than the triamine N-3-N-3-N.

The titration curves of Figures 1-3 have some common features. Thus, it is seen that at low values of N/P the ^{23}Na relaxation rate decreases rapidly and approximately linearly with N/P. Then follows a middle region where the curves are more complex but in many of them there are "humps" which are reproducible. Finally, there is a ten-

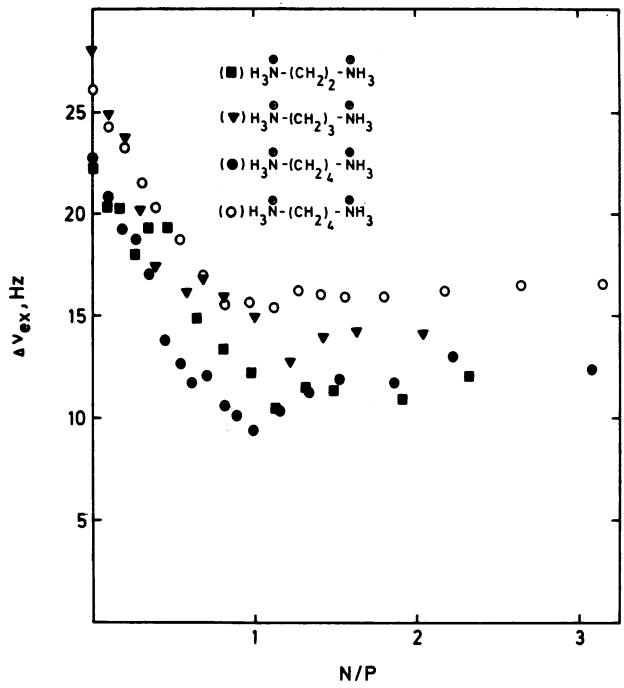

Fig. 3 - The excess ^{23}Na linewidth as a function of added diamine in a solution of DNA, diamine and sodium phosphate.
○, diaminobutane (putrescine). DNA was 6.4 mM, Na phosphate was 10 mM (21.4 mM total Na^+). ●, diaminobutane. DNA was 13.5 mM, Na phosphate was 30 mM (58.5 mM total Na^+). ▼, diaminopropane. DNA was 16.6 mM, Na phosphate was 30 mM (61.6 mM total Na^+). ■, diaminoethane. DNA was 13.1 mM, Na phosphate was 30 mM (58.1 mM total Na^+).

dency for some of the curves to level off at increasing N/P. The behaviour is complex and not easily interpreted. The initial slope of the curves for the polyamines N-3-N-4-N (spermidine) N-3-N-2-N-3-N and N-3-N-4-N-3-N (spermine) is close to that expected if each charged amino or imino group were releasing one sodium ion from the DNA polyanion. This type of behaviour is predicted by Manning's theory of counterion condensation in the limit of low ionic strength[34-36] (cf. also refs. 9 and 37): a polyvalent cation ion of charge Z displaces Z monovalent cations from a polyanion. Only the two naturally occurring polyamines spermine and spermidine come close to expelling all sodium ions from DNA at N/P ~ 1, as indicated by the pronounced reduction in

the $^{23}Na^{+}$ relaxation rate. By contrast for the tetraamine N-3-N-2-N-3-N, which differs from spermine by two $-CH_2-$ groups in the central polymethylene chain, the relaxation rate levels off at roughly 40-45% of its initial relaxation rate. This behaviour is most unexpected - it would appear that above an N/P ratio of about unity none of the diamines nor the triamine N-3-N-3-N nor the tetraamine N-3-N-2-N-3-N are able to compete with sodium ions still associated with DNA. Furthermore addition of excess diamine e.g. diaminobutane to a solution of one of the other diamines e.g. diaminopropane and DNA (N/P~ 2) had little or no effect on $\Delta\nu_{ex}$, leaving it around 50% of its initial value.

Marked differences also occur between polyamines in precipitation behaviour. Thus at N/P ratios of ~1 for spermine and ~2 for spermidine the DNA-polyamine complex precipitated irreversibly. Precipitation was not found to occur for any of the other polyamines studied even at high (>2) N/P ratios. However, addition of Mg^{2+} (Mg^{2+}/P~0.1) to solutions of diamines and DNA(N/P~ 2) produced irreversible precipitation of the diamine-DNA complex. As Mg^{2+} in the absence of diamine does not precipitate DNA, this may reflect a conformational change in DNA on diamine binding.

Finally, using ^{23}Na NMR we find no indication of significant putrescine binding to single stranded (heat denatured) calf thymus DNA and very little binding to DNA at pH 8.25. The latter result is interesting in the light of e.m. studies which indicate that DNA compacted by spermidine is unfolded in 0.12 M Na^{+} at pH 8.0[6].

It should be evident from the present experimental data that the interaction of polyamines with DNA is a complex phenomenon and can not be rationalized without taking the detailed molecular structure of the polyamine into account. In this respect our results do not argue against the models for DNA-polyamine complexes referred to in the Introduction. It would also appear, however, that the models published so far are incomplete and unable to explain the ^{23}Na relaxation data. The structure of the polyamine-DNA complex (or complexes) at N/P ratios approaching unity must in all likelihood be considered. Also the possibility that polyamines may cause conformational changes of the DNA molecules (cf. ref. 9) must be taken into account.

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