Isopycnic sedimentation of DNA in metrizamide: the effect of low concentrations of ions on buoyant density and hydration

G.D. Birnie, Elizabeth MacPhail and D. Rickwood

The Beatson Institute for Cancer Research, 132 Hill Street,
Glasgow G3 6UD, Scotland.

Received 17 June, 1974

ABSTRACT

Metrizamide, an inert, non-ionic organic compound, dissolves in water to give a dense solution in which DNA bands isopycnically at a density corresponding to that of fully hydrated DNA. Density-gradient centrifugation in solutions of metrizamide has been used to determine the effects of very dilute solutions of salts on the buoyant density of native and denatured DNA. It has been shown that the buoyant density of DNA is dependent on both the counter-cation and the anion present. Interpretation of the data in terms of the degree of hydration of the macromolecule indicates that (i), NaDNA is more highly hydrated than CsDNA; and (ii), the hydration of NaDNA varies with anion in the order sulphate < fluoride < chloride < bromide < iodide.

It is suggested that isopycnic centrifugation in metrizamide is a simple method for determining the effects of salts (and other small molecules) on the hydration of nucleic acids under conditions of high ratios of salt to DNA ($> 5 \times 10^3$ moles/mole) while high (0.999) water activity is maintained.

INTRODUCTION

DNA exists in solution as a hydrated polyanion and it is as such that DNA interacts with other molecules of biological importance. Thus the extent to which DNA is hydrated is of some consequence, particularly since the rate and extent of the interaction of DNA with, say, a protein may well be influenced by changes in the hydration of the DNA. Many different techniques have been used to study the hydration of DNA, and the effects of concentration and species of salts and other small molecules, on the interaction of DNA with water. However, the values obtained vary with the methods by which they were determined since some methods measure only tightly bound water whereas others include in the estimate those water molecules which are more loosely associated with the DNA. For example, Chatteraj and Bull¹ have shown that, in concentrated solutions of salts, the hydration of DNA is very low and is influenced both by the cations and anions present. In contrast, Hearst² concluded, from experiments done with more dilute salt

solutions, that DNA is highly hydrated but that the extent of hydration is predominantly influenced by water activity and is not very sensitive to the anion present in the solution. However, for a number of technical reasons, current methods do not allow accurate estimates to be made of the influence of salts on the hydration of DNA under conditions where the concentrations of both the DNA and the salts are low; that is, conditions where DNA concentration is very low so that high ratios of salt to DNA can be obtained while water activity is maintained at values as high as 0.999.

Recently, a new buoyant density-gradient solute, metrizamide, has been introduced and has proved useful in experiments involving the isopycnic sedimentation of nucleoproteins³⁻⁶. Metrizamide is an inert, non-ionic organic compound which dissolves in water to give dense solutions of relatively low viscosity. Moreover, nucleic acids band isopycnically in low concentrations of metrizamide (0.2 to 0.5 M), and display buoyant densities which correspond to those of fully hydrated molecules. This observation suggested that isopycnic sedimentation in metrizamide might be applicable to studies of the hydration of DNA and the interaction of salts and other small molecules with DNA under those conditions for which current methods are not suitable. This paper presents the results of preliminary experiments which suggest that the technique can be used to demonstrate the effect of low concentrations of cations and anions on the hydration of DNA.

EXPERIMENTAL

NaF, NaBr, NaCl, Na₂SO₄, CsCl and Cs₂SO₄ were Analar grade salts purchased from British Drug Houses, Poole, Dorset; 0.5 M solutions were prepared in 50 mM HEPES-NaOH, 5 mM EDTA, pH 7.5. NaI was reagent grade purchased from Hopkins and Williams Ltd., Chadwell Heath, Essex; a 0.5 M solution was prepared in the same buffer to which 5 mM Na₂SO₃ was added to inhibit oxidation of I⁻. Heavy metal ions were removed by passing the solutions (100 ml) through a 10 ml bed of Chelex-100 (BioRad Laboratories), the first 20 ml of the effluent being rejected. All water used for diluting these solutions was passed through a mixed-bed deionizer, glass-distilled then passed through Chelex-100.

NaDNA labelled with [2-¹⁴C]thymine was prepared from mouse IS cells as described previously⁵ and dissolved in 10 mM NaCl. To equilibrate the DNA with the salts, 0.1 ml portions containing 20 µg of DNA were mixed with 1 ml portions of the 0.5 M buffered salt solutions and left

at 20° overnight. These solutions were then passed through columns (25 cm x 0.9 cm) of Sephadex G-25 which had previously been equilibrated with a 1:25 dilution of the appropriate stock salt solution, elution being with the same dilution of the salt solution.

Metrisamide [2-(3-acetamido-5-N-methylacetamido-2,4,6-tri-iodobenzamido)-2-deoxy-D-glucose] was a generous gift from Nyegaard and Co. A/S, Oslo, Norway. It was dissolved in 1 mM HEPES-NaOH, 1 mM-EDTA, pH 7.5 and mixed with [¹⁴C]DNA and the appropriate stock salt solution. The density of the mixture was adjusted to that required to ensure that the DNA banded at, or near, the middle of the gradient⁴ and 5 ml portions (containing 5 µg of DNA), overlaid with paraffin, were centrifuged in 10 ml polyallomer tubes in the aluminium 10 x 10 fixed-angle rotor of an MSE superspeed 50 ultracentrifuge at 30,000 rev/min for 68 h at 5°. The gradients were fractionated by upwards displacement⁴, and the density of each 0.25 ml fraction was calculated from its refractive index, after correction for the salt present, using the relationship

$$\rho_{5^{\circ}} = 3.4533 \rho_{20^{\circ}} - 3.601$$

The remainder of each fraction was mixed with 0.8 ml of water and 10 ml of a Triton X-100, toluene-based scintillation fluid, and its radioactivity measured by liquid scintillation spectrometry.

RESULTS

Since the water activity of very dilute solutions of different salts, as calculated from freezing point depression data, do differ slightly, the effect of very small changes in water activity on the buoyant densities of DNA was first determined by centrifuging DNA to equilibrium in metrisamide gradients which contained different concentrations of NaCl. Table 1 shows the changes observed in the buoyant density of native and denatured mouse DNA when the concentration of NaCl in the gradients was varied from 20 mM to 204 mM. If these changes in buoyant density are due solely to changes in hydration of the DNA, it is clear that the decrease in water activity over this range in salt concentrations has only a small, though still significant, effect on the hydration of native and denatured DNA. Moreover, the effect is only significant when the water activity is reduced below about 0.997 and, therefore, it can be presumed that any slight differences among the water activities of 20 mM solutions of different salts will have an insignificant effect on the hydration of

Table 1: The effect of water activity on the buoyant density and hydration of mouse DNA.

Concentration of NaCl (mM)	a_w^a	native DNA		denatured DNA	
		buoyant density (g/cm ³)	moles H ₂ O per mole nucleotide	buoyant density (g/cm ³)	moles H ₂ O per mole nucleotide
20	0.9990	1.119	68	1.137	58
96.8	0.9968	1.123	66	1.142	56
204	0.9932	1.128	63	1.151	52

^a Calculated from freezing point depression data¹⁰

Table 2: The effect of Cs⁺ and Na⁺ on the buoyant density and hydration of native DNA in 20 mM salt.

Salt present	$\bar{v}_2^a$ (cm ³ /g)	buoyant density (g/cm ³)	hydration (moles H ₂ O per mole nucleotide)
NaCl	0.500	1.119	68
CsCl	0.440	1.179	49
Na ₂ SO ₄	0.500	1.133	60
Cs ₂ SO ₄	0.440	1.200	45

^a From Cohen and Eisenberg⁷

Table 3: The effect of anions on the buoyant density and hydration of DNA in 20 mM salt.

Salt present	native DNA		denatured DNA	
	buoyant density (g/cm ³)	moles H ₂ O per mole nucleotide	buoyant density (g/cm ³)	moles H ₂ O per mole nucleotide
Na ₂ SO ₄	1.133	60	1.159	49
NaF	1.126	64	1.158	49
NaCl	1.119	68	1.137	58
NaBr	1.109	75	1.130	62
NaI	1.100	83	1.114	71

DNA. For this reason, all determinations of the effect of a salt on the buoyant density of DNA were done with this concentration of the salt incorporated in the density gradient.

The effect of a change in counter-ion on the buoyant density of DNA was examined by comparing the buoyant densities of CaDNA and NaDNA. The results are summarised in Table 2. Since there is a marked difference between the partial specific volumes of CaDNA and NaDNA⁷, it was to be expected that the buoyant densities of these salts of DNA would also differ markedly in metrisamide gradients. However, the buoyant densities of CaDNA and NaDNA differ to a greater extent than can be accounted for simply by the difference between their partial specific volumes. When this difference is taken into account it appears that CaDNA is considerably less hydrated than NaDNA and, moreover, that the hydration is decreased by the same amount in both chloride and sulphate solutions.

Table 2 also shows that the buoyant densities of both CaDNA and NaDNA are lower in the presence of chloride ions than in the presence of sulphate ions. This suggested that anions, as well as cations, may have a significant effect on the net hydration of DNA. Accordingly, the influence of anions on the hydration of DNA was examined in more detail by comparing the buoyant densities of NaDNA in the presence of Na₂SO₄, NaF, NaCl, NaBr and NaI. The data summarised in Table 3 clearly show that a change in anion can cause a large change in the buoyant density of NaDNA, and that these changes are particularly striking when expressed in terms of the net hydration of the DNA. The buoyant densities of both native and denatured DNA are influenced by these anions, though it is apparent from Table 3 that the relative effects of the anions on native and denatured DNA do differ to some extent.

DISCUSSION

Metrisamide has only recently been introduced as a buoyant density-gradient solute, and its properties have yet to be fully elucidated. However, the evidence available so far indicates that metrisamide does not interact with DNA⁵, though it does form labile complexes with some proteins⁸. In addition, the extremely low buoyant densities of DNA and RNA in metrisamide gradients suggest that nucleic acids are fully hydrated in metrisamide⁵, thus indicating that, at the low concentration used (<0.5 M), the hydration of metrisamide is too low to have a significant effect on water activity.

The data presented here clearly show that the counter-ion in a DNA solution has a marked effect on the buoyant density of the DNA over and above that which can be accounted for by its effect on the partial specific volume of the DNA. This effect is most readily interpreted in terms of the hydration of the DNA. Support for this interpretation is provided by Cohen and Eisenberg⁷, who showed that, in dilute salt solutions, the hydration of CsDNA is significantly lower than that of NaDNA. In addition, the hydration of CsDNA and NaDNA, calculated from their buoyant densities in metrizamide, agree quite closely with the values determined in dilute salt solutions by Cohen and Eisenberg⁷. The difference in hydration of CsDNA and NaDNA (17-19 moles of water per mole of nucleotide) is large, and appears much too large to be due only to differences in the extent of hydration of the cations. If this is so, cations must influence the interaction of DNA and water by some other mechanism. One possible explanation comes from the work of Dix and Straus⁹, who have shown that the thermal stability of native DNA is a function of the counter-ion present, and have interpreted their data in terms of differences in the degree to which different cations affect the structure of water in the vicinity of DNA molecules, with consequent effects on the extent of the interaction of DNA with water.

Anions also have a significant effect on the buoyant density of DNA. Although it cannot be ruled out that the differences observed are due to differential interaction of anions with DNA (which might alter the partial specific volume of DNA), no such interaction has been observed in very dilute solutions of salts. A likely explanation of the data is that the buoyant density reflects the hydration of the DNA and, therefore, that anions have a significant effect on the extent of this hydration. This could arise from differential effects of anions on the interaction between water molecules and DNA. The observation that the hydration of DNA, as calculated from its buoyant density in metrizamide, varies with species of anion in the order $\text{SO}_4^{2-} < \text{F}^- < \text{Cl}^- < \text{Br}^- < \text{I}^-$ lends support to this suggestion, since it is in this order that these anions occur in the Hofmeister series.

The observations reported in this paper suggest that isopycnic sedimentation in density gradients of metrizamide is a simple technique which holds some promise for studies of the hydration of, and the interaction of ions with, nucleic acids under conditions in which the methods currently available are least accurate and sensitive, namely,

low concentrations of ions and nucleic acids. In addition, this technique can be used for studies of the interaction of small molecules with DNA or RNA, particularly when such interactions lead to changes in the conformation of the nucleic acids with consequent changes in their hydration.

ACKNOWLEDGMENTS

This work was supported by grants to the Beatson Institute from the Medical Research Council and the Cancer Research Campaign; metrizamide was a generous gift from Nyegaard and Co., A/S. We are grateful to Drs. John Paul, R. Eason and Ailsa Campbell for useful, critical discussion.

REFERENCES

- 1 Chatteraj, D.K. and Bull, H.B. (1971) *Archiv. Biochem. Biophys.* 142, 363-370.
- 2 Hearst, J.E. (1965) *Biopolymers* 3, 57-68.
- 3 Mullock, R.M. and Hinton, R.H. (1973) *Biochem. Soc. Transact.* 1, 579-581.
- 4 Rickwood, D., Hell, A. and Birnie, G.D. (1973) *FEBS Lett.* 33, 221-224.
- 5 Birnie, G.D., Rickwood, D. and Hell, A. (1973) *Biochim. Biophys. Acta* 331, 283-294.
- 6 Rickwood, D., Hell, A., Malcolm, S., Birnie, G.D., MacGillivray, A.J. and Paul, J. (1974) *Biochim. Biophys. Acta* (in press).
- 7 Cohen, G. and Eisenberg, H. (1968) *Biopolymers* 6, 1077-1100.
- 8 Rickwood, D., Hell, A., Birnie, G.D. and Gilhuus-Moe, C.C. (1974) *Biochim. Biophys. Acta* 342, 367-371.
- 9 Dix, D.E. and Straus, D.B. (1972) *Archiv. Biochem. Biophys.* 152, 299-310.
- 10 Wolf, A.V. and Brown, M.G. (1971) in *Handbook of Chemistry and Physics*, 51st edn., pp. D176-D215.