Eur Biophys J (1987) 15: 59–63

European Biophysics Journal

Hydration properties of DNA-lysine gels by microwave dielectric measurements as a function of temperature

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Received October 6, 1986/Accepted in revised form March 17, 1987

Abstract. Dielectric measurements by a cavity perturbation method at 10 GHz in the temperature range from −20 °C to +45 °C are reported for aqueous gels of herring sperm DNA in the presence of 1 or 3 lysine molecules per nucleotide. Measurements for lysine-water and DNA-water systems are also reported. The experimental results can be accounted for by the presence of interfacial water, with dielectric properties different from those of bulk water, and are analyzed in terms of a three component equation (solute molecules, interfacial water and bulk water) to calculate hydration parameters of the systems. The lysine molecule is found to coordinate a particular number of water molecules, in agreement with the literature. The specific hydration of DNA is reduced by the presence of lysine, indicating a direct interaction between the polyion and the aminoacid: a decrease to about 50% was observed at a ratio of one molecule of lysine per nucleotide. A suggestion is made that the interaction is mainly electrostatic in nature.

Key words: DNA hydration, DNA-lysine interaction, DNA gel, microwave dielectric properties

Introduction

Dielectric measurements at microwave frequencies on NaDNA-water systems are a valid tool in studying the interaction between the polyion and water. In a recent work (Bonincontro et al. 1986) we reported a study on the hydration of NaDNA gels performed by dielectric measurements at 10 GHz in a wide range of temperatures (from −15 °C to +45 °C). We estimated a total number of about 35 water molecules per nucleotide interacting with DNA, including very strongly and less strongly bonded molecules. The value of specific hydration we determined is in good agreement with experimental and theoretical data available in the literature. The same technique enables us to study the modification of DNA hydration that occurs when the polyion interacts with other molecules.

In this paper we report an investigation on the hydration of DNA in the presence of lysine molecules. A comparison of the hydration parameters in the water-lysine-DNA, water-lysine and water-DNA systems points to a direct interaction between DNA and lysine.

We take an understanding of the interaction between DNA and a single aminoacid such as lysine as a first step in the approach to more complicated systems like DNA-protein complexes.

Materials and methods

NaDNA from herring sperm was from Boehringer. The DNA content of the lyophilized product was 95% (w/w), as estimated by phosphorous analysis (Knight and Woody 1958); RNA and protein contaminations were found to be less than 1% (w/w), as determined by the orcinol method (Schneider 1957), and 0.3% (w/w), as determined by the method of Lowry et al. (1951), respectively. The size distribution of NaDNA molecules was in the range of 1.1 to 0.3 kilobases, as determined by agarose gel electrophoresis (Sudgen et al. 1975). The NaDNA was usually lyophilized further to constant weight and dissolved in 10^{-3} *M* NaCl.

Lysine monohydrochloride, analytical grade, was from Carlo Erba. The lyophilized powder was dissolved at the desired concentration by weighing.

Densities of anhydrous NaDNA and Lysine were assumed to be 1.9 g/cc (Wang 1955) and 1.4 g/cc respectively.

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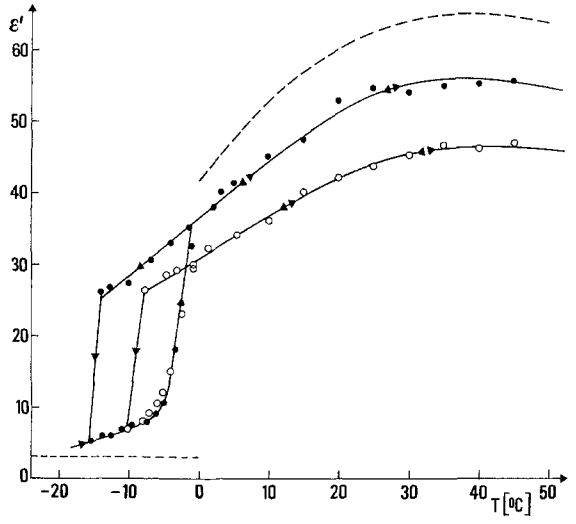

Fig. 1.

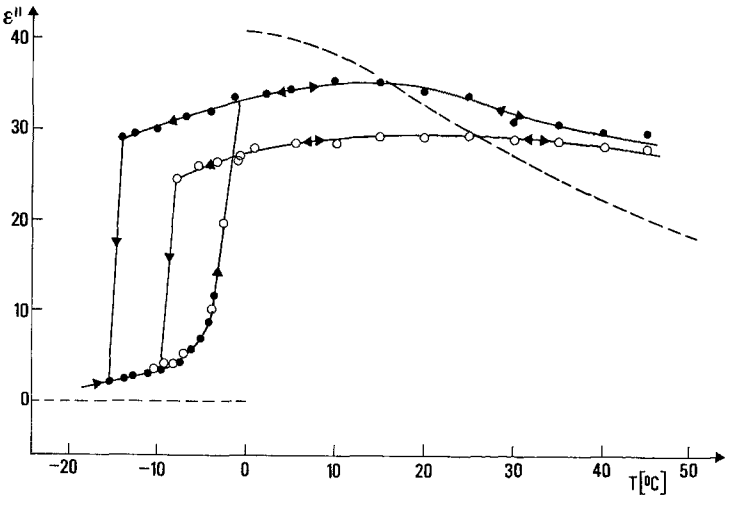

Fig. 2.

Fig. 1. Permittivity ε' at 10 GHz of: (●) a water-lysine solution (0.54 *M* lysine), (○) a water-lysine-DNA system (0.49 *M* lysine and 0.48 *M* DNA) as a function of the temperature. Permittivity of water (*dotted line*) is also reported

Fig. 2. Dielectric loss ε'' at 10 GHz of: (●) a water-lysine solution (0.54 *M* lysine), (○) a water-lysine-DNA system (0.49 *M* lysine and 0.48 *M* DNA) as a function of the temperature. Dielectric loss of water (*dotted line*) is also reported

Dielectric measurements were performed using a microwave cavity perturbation method. Details of the method employed are reported elsewhere (Bonincontro and Cametti 1971, 1977). Briefly, a resonant cylindrical cavity harbored along its symmetry axis a constant section of the sample which was passing through both bases. The cavity was oscillating in the TE_{011} mode at the frequency of 10 GHz and ε', ε'' were obtained from variation of the resonance frequency and Q factor of the cavity respectively. The choice of the resonant cavity allows the use of small volumes of samples (below 100 µl), which can be contained in a glass capillary easy to assemble in the cavity. Accuracy on ε' and ε'' determinations were within 2% and 1% respectively. The measurements were performed in the temperature interval from −20 °C to +45 °C. The temperature of the sample was controlled within ± 0.1 °C.

Results and discussion

The permittivity ε' and the dielectric loss ε'' of NaDNA aqueous gels in the presence of lysine have been measured at a frequency of 10 GHz in the temperature range from −20 °C to +45 °C. Similar measurements were previously performed on aqueous gels of NaDNA (Bonincontro et al. 1986). NaDNA and lysine concentrations were chosen so as to have the same number of water molecules per nucleotide in all the samples and two different ratios (1 : 1 and 3 : 1) of lysine molecules to DNA nucleotides. Reference spectra of aqueous solutions of lysine at the concentrations used for DNA gel preparations were also recorded.

Figures 1 and 2 show the temperature dependence of ε' and ε'' respectively in a 0.54 *M* lysine solution and in the gel obtained by addition of NaDNA up to 0.48 *M* (nucleotide).

Figures 3 and 4 show the temperature dependence of ε' and ε'' respectively in a 1.62 *M* lysine solution and in the corresponding gel containing 0.42 *M* DNA (nucleotide).

The dielectric behavior of the samples investigated is very different from that of the solvent alone. Relevant hysteresis cycles are present below the freezing point of the bulk water. Similar phenomena have been reported in dielectric measurements on different biological samples (Gabriel and Grant 1985). In our systems we observe that the extension of the hysteresis cycle is decreased by the presence of DNA. We believe that a thermodynamic approach would be suitable to a better understanding of the physico-chemical properties of supercooled water in these systems.

Aminoacids like lysine, with a dipolar moment of about 20−30 D, are known to exhibit a temperature dependent Debye dielectric relaxation centered at frequencies of the order of 10^2 MHz (Shepherd and Grant 1968). However, at 10 GHz and at tem-

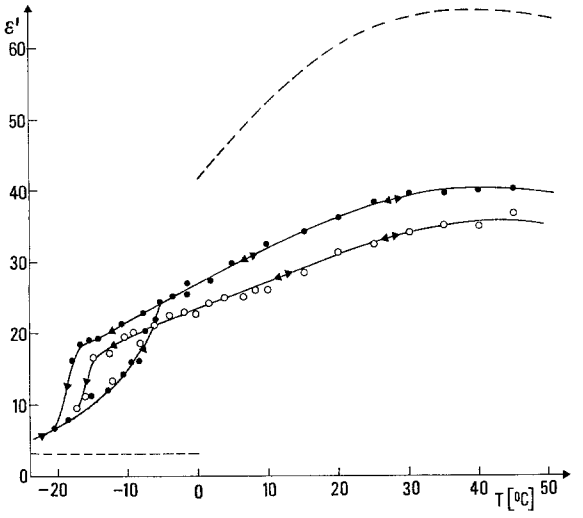

Fig. 3.

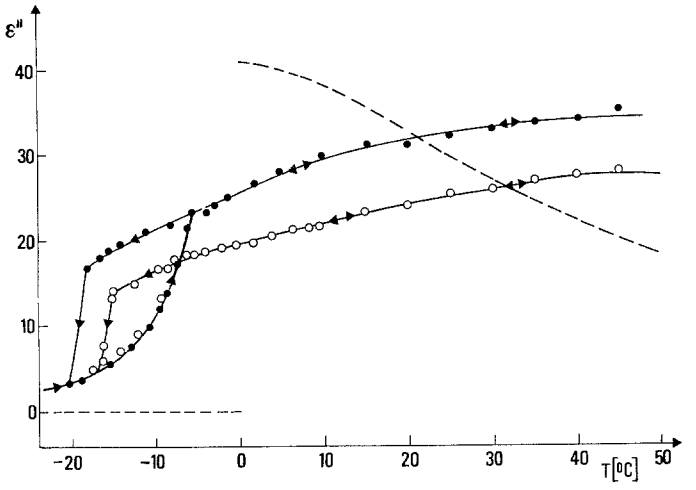

Fig. 4.

Fig. 3. Permittivity ε' at 10 GHz of: (●) a water-lysine solution (1.62 *M* lysine), (○) a water-lysine-DNA system (1.49 *M* lysine and 0.42 *M* DNA) as a function of the temperature. Permittivity of water (*dotted line*) is also reported

Fig. 4. Dielectric loss ε'' at 10 GHz of: (●) a water-lysine solution (1.62 *M* lysine), (○) a water-lysine-DNA system (1.49 *M* lysine and 0.42 *M* DNA) as a function of the temperature. Dielectric loss of water (*dotted line*) is also reported

peratures not exceeding 20 °C, the dipolar polarization mechanism can be assumed to be over for lysine, and contributions to the dielectric constant come only from atomic and electronic polarization; this holds even more in the case of polyions like DNA. Under these conditions the dielectric constant of lysine and DNA may be assumed real and independent of temperature. A reasonable value for the permittivity of both DNA and lysine is 2.5, as is commonly reported in the literature for biological systems (e.g. Pennock and Schwan 1969), and as can be calculated from the atomic polarization values (Pethig 1979).

We examined the experimental data in the light of a few mixture formulae. In particular, we applied the Wagner formula, the Bruggeman formula and the Böttcher formula (Hasted 1973). Furthermore, we considered a model of a two-phase water suspension derived by Kraszewski et al. (1976). This model considers alternating layers of dispersed phase and water, whose thickness is small in comparison with the employed wavelength, neglecting the reflection at interfaces. Under these assumptions, the authors report the following formula for the dielectric properties of heterogeneous systems with a dispersed phase of irregular shape:

$$(\varepsilon^*)^{1/2} = p_i(\varepsilon_i^*)^{1/2} + p_w(\varepsilon_w^*)^{1/2} \qquad (1)$$

where ε^*, ε_i^*, ε_w^* are the complex dielectric constants of the system, the dispersed phase and the water, respectively; p_i and p_w are the fractional volumes of the dispersed phase and the water, respectively ($p_i + p_w = 1$). Equation (1), valid for all frequencies and temperatures, has been applied to a clay-sand slurry suspended in water, at microwave frequencies, and excellent agreement with experimental results has been obtained for a wide range of dispersed-phase concentrations in the temperature range 6° – 60 °C (Kraszewski et al. 1976). On the other hand, Eq. (1) gives values very close to those calculated with the other mixture formulae for our samples. No mixture formula for two component systems can fit our experimental data. Therefore, we must consider the presence of interfacial water as a third component, with dielectric properties different from those of bulk water.

A suitable theoretical approach to the study of the dielectric properties of our systems is the application of the Kraszewski equation extended to a three component system (solute molecules, interfacial water and bulk water):

$$(\varepsilon^*)^{1/2} = p_i(\varepsilon_i^*)^{1/2} + p_h(\varepsilon_h^*)^{1/2} + p_w(\varepsilon_w^*)^{1/2}, \qquad (2)$$

where ε^* is the complex dielectric constant of the system; ε_i^*, ε_h^* and ε_w^* are the complex dielectric constants and p_i, p_h and p_w the fractional volumes of the three components respectively. It must be noted that the application of the Wagner or similar formulae to three component systems implies the use of an iterative calculation, resulting in lower accuracy of the fitting procedure.

Table 1. Hydration parameters of DNA-lysine systems

Sample	Solute molarity		Modified water fract. volume	Bound water molecules	
(W = water)	Lys	DNA	p_h [%]	Out of total water molecules Ø [%]	Per solute molecule
W-DNA		0.51 ± 0.01	30 ± 4	33 ± 5	35 ± 5
W-Lys	0.54 ± 0.01		20 ± 3	22 ± 3	20 ± 3
W-Lys	1.62 ± 0.01		50 ± 5	63 ± 8	17 ± 4
W-Lys-DNA	0.49 ± 0.01	0.48 ± 0.01	45 ± 5	53 ± 6	26 ± 4
W-Lys-DNA	1.49 ± 0.01	0.42 ± 0.01	65 ± 5	89 ± 8	19 ± 3

The experimental data are difficult to analyze, possibly because *i)* the binding between solute molecules and water, and among solute molecules is affected by temperature, and *ii)* the Debye polarization of lysine at temperatures above 20 °C may contribute to the dielectric constant of the system. These difficulties are overcome if the experimental data are analyzed in a restricted temperature interval around the freezing point of bulk water in the sample. Also, the fractional volume p_h of interfacial water can be assumed constant within this interval. For a fixed value of the p_h parameter we calculated ε_h' and ε_h'' of the modified water. We imposed the condition that the values of ε_h' and ε_h'' obtained below and above the freezing point of bulk water (calculated on the basis of the cryoscopic depression) must coincide at this temperature. The best value for the fractional volume of the modified water was therefore obtained by means of a polynomial regression analysis.

The number of water molecules hydrating the solute molecules, obtained by the fitting procedure, are reported in Table 1. For comparison, the values of specific hydration of the DNA obtained in an earlier work are also reported in the same table. As we have pointed out (Bonincontro et al. 1986), we measure a number of water molecules interacting with the polyion that includes both strongly bonded molecules and less strongly bonded (groove) molecules. Our results for the DNA alone are in good agreement with theoretical predictions (Clementi and Corongiu 1982) and with experimental data on water strongly bonded to DNA (Cross and Pethig 1983). In all the samples investigated free water is always present (see Ø values in Table 1). The values obtained for the number of bound water molecules per solute molecule (last column of Table 1) clearly show that the specific hydration of DNA is reduced by the presence of lysine. Furthermore, it must be considered that lysine itself is able to coordinate a particular number of water molecules: our experimental value (about 20 water molecules per lysine molecule) is in very good agreement with the literature (Hollenberg and Ifft 1982). The hydration numbers estimated for the water-lysine-DNA systems thus indicate that DNA and lysine have reduced their solvation shells by at least 50%–60%. This suggests a direct binding of lysine to DNA, accompanied by exclusion of bound water molecules.

The dielectric analysis in our conditions cannot yield accurate determinations of dielectric relaxation times, hence no estimation of the interaction energies involved can be derived.

Some information about the DNA-lysine interaction may be obtained by a ligand displacement assay. Preliminary data from solution spectroscopy with ethidium bromide indicate that lysine decreases the association constant of the drug to DNA, while the number of binding sites is not affected (unpublished results). This effect can be explained in terms of electrostatic interactions, as in the case of cations like Na^+ and Mg^{++} (Waring 1965). It is well known that rearrangements of the structure of the DNA hydration shell can be induced by the presence of different cations which bind to the backbone phosphates (Clementi and Corongiu 1982). We have observed a marked reduction in the specific hydration of DNA in the presence of at least one molecule of lysine per nucleotide. On these grounds we can assume that lysine interacts mainly with the phosphate groups of DNA, displacing water molecules.

Acknowledgements. This work was supported in part by a grant from the "Ministero della Pubblica Istruzione" of Italy.

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