Biochemical Pharmacology, Vol. 37, No. 9, pp. 1839–1840, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

HYDRATION OF AMINOACID-DNA AND PROTEIN-DNA SYSTEMS.

A. Bonincontro[a], R. Caneva[b,c], F. Pedone[c]

a) Dpt Fisica. GNSM and CISM; b) CS Acidi Nucleici CNR;

c) Dpt Genet. Biol. Mol.

Universita' Roma I

We are studying the specific hydration of aqueous DNA gels by dielectric spectroscopy at microwave frequency. Determinations of the real and the imaginary part of the dielectric constant are performed by a cavity perturbation method at 10 GHz in a wide range of temperatures below and above the freezing point of bulk water (from -20 °C to +45 °C) [1]. The comparison between experimental data and theoretical values computed by a suitable mixture formula [2] allows us to estimate the amount of water which is modified by the interactions with solute molecules.

In a recent work we applied this procedure to the study of aqueous DNA gels in the presence of lysine [3]. In such a system the hydration value per solute molecule (DNA nucleotides plus lysine molecules) is not additive, on the contrary it is greatly reduced (20-25 bound water molecules) even with respect to the hydration of the DNA alone (35 bound water molecules). We interpreted this result as an indication of a strong electrostatic interaction accompanied by a release of water molecules; this interpretation was supported by ethidium bromide displacement assays.

In view of the relevance of basic proteins to the condensation of nucleic acids, and in particular in view of the role of protamines, small very arginine-rich extremely basic proteins of the sperm nucleus, we extended our experimental approach to arginine-DNA and clupeine-DNA systems. Clupeine was selected to ensure the homology of protamine and DNA sources (from herring sperm). Solutions of arginine and clupeine respectively were also investigated as reference samples.

The experimental results (ε', ε'' as a function of temperature at 10 GHz) are reported in figures 1, 2 for an aqueous DNA gel in the presence of clupeine; similar curves were obtained for the other samples.

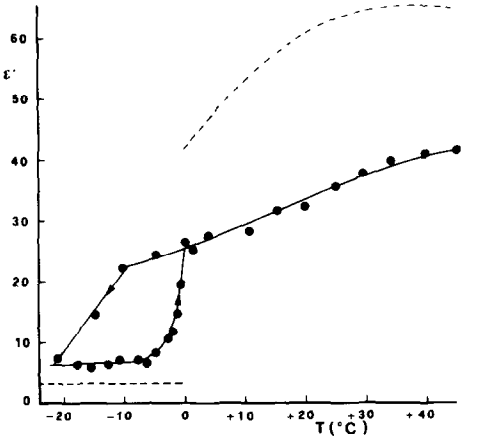

Fig. 1. Permittivity ε' of a water-DNA-clupeine system at a 1:1 nucleotide to aminoacid ratio (0---0) and water (- - -).

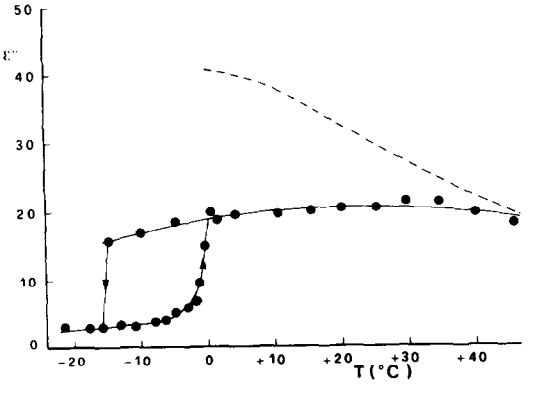

Fig. 2. Dielectric loss ε'' of a water-DNA-clupeine system at a 1:1 nucleotide to aminoacid ratio (0---0) and water (- - -).

TABLE 1. Hydration parameters of DNA-Arginine and DNA-Clupeine systems.

sample	solute molarity: DNA[P]	solute molarity: aminoacid	modified water fract. volume (%)	bound water mol. per solute mol. (%)
W-DNA	.51 ± .01		30 ± 4	35 ± 5
W-Arg		1.58 ± .03	33 ± 5	12 ± 2
W-DNA-Arg	.39 ± .01	1.47 ± .03	75 ± 10	22 ± 3
W-DNA-Arg	.39 ± .01	.48 ± .01	32 ± 5	21 ± 3
W-Clup		5.1* ± .1	25 ± 4	3* ± 1
W-Clup		.105* ± .003	≤ .5	≤ 3*
W-DNA-Clup	.47 ± .01	.65* ± .01	45 ± 7	22* ± 3

* The clupeine is considered in terms of its aminoacid residues.

The dielectric behavior of the samples investigated are very different from that of the solvent alone. Moreover, 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 [4].

The hydration parameters were derived from the curves according to the procedure described [3] and are presented in table 1.

The effect of arginine on the specific hydration in the water-DNA-aminoacid system is substantially equivalent to that of lysine, resulting in a 50-60 % reduction with respect to the sum of hydrations of the components. On the contrary, the specific hydration of arginine in solution is found to be significantly lower than that of lysine (12 against 20 bound water molecules). This fact suggests that the decrease of specific hydration in DNA-arginine gels may affect mainly the nucleic acid component. Identical hydration values for DNA-arginine systems were obtained with two different aminoacid contents, one of which gives no free water molecules.

The specific hydration of clupeine in solution, as expressed per aminoacid residue, is found to be extremely lower than that of free arginine, as can be expected from a compact configuration assumed by the protein in solution. However, in the case of DNA gels, the effect of clupeine on the specific hydration, as expressed per nucleotide plus aminoacid residue molecules, is identical, within the experimental error, with that found in the case of arginine. This result is consistent with nucleoprotamine models in which the protein molecules undergo an extended conformation while winding around DNA double helices [5, 6]. As far as the specific hydration is concerned, our results suggest that arginine residues in the clupeine molecule behave like free arginine molecules in the interactions with DNA.

REFERENCES

1. A. Bonincontro, A. Di Biasio and F. Pedone, Biopolymers 25, 241 (1986).
2. A. Kraszewski, S. Kulinski and M. Matuszeswki, J. App. Phys. 47, 1275 (1976).
3. A. Bonincontro, R. Caneva and F. Pedone, Europ. Biophys. J., in press.
4. C. Gabriel and E. H. Grant, Phys. Med. Biol. 30, 975 (1985).
5. R. W. Warrant and S. H. Kim, Nature 271, 130 (1978).
6. I. Fita, J. L. Campos, L. C. Puigjaner and J. A. Subirana, J. Mol. Biol. 167, 157 (1983).