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On the Thermal Stability of DNA in Solution of Mixed Solvents

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Abstract. The study of the changes in UV absorbance of DNA solutions in water/dioxane and water/ethylene glycol mixture at different concentrations shows that the thermal denaturation of DNA is sensitive to the electrical permittivity of the media and the water content. At relative low concentrations of co-solvent the dominant feature is the electrical permittivity. When water content is lower than a critical value, the electrical permittivity is no longer the determinant of the denaturation temperature but the partial volume fraction of water. The critical water content is about 0.69 partial volume fraction of water.

Key words: DNA stability, hydrophobic interactions, water stabilization effects, electrostatic interactions in DNA.

1. Introduction

One of the simple but yet effective ways to study the thermal denaturation of DNA is the measurement of the increment of UV absorbance of solutions. There is a straightforward relationship of the degree of denaturation, the helix content, and the UV absorbance. This relationship is related to the absorption of purine and pyrimidine base around 260 nm. When the denaturation proceeds the bases are released from their double helical stacking increasing the absorbance accordingly [1,2].

In a previous work [3] we have shown that there is a linear relationship between the changes in the screening of charges due to the electrical permittivity of the solution and the melting temperature of DNA, irrespective of the nature of the solvent, at least up to some concentration value for the studied solvents.

In this work we have extended the study to higher concentrations of dioxane and ethylene glycol showing that, after a critical value of water content, the permittivity is no longer the determinant of the melting temperature and it becomes dependent on the partial volume of water in the solution.

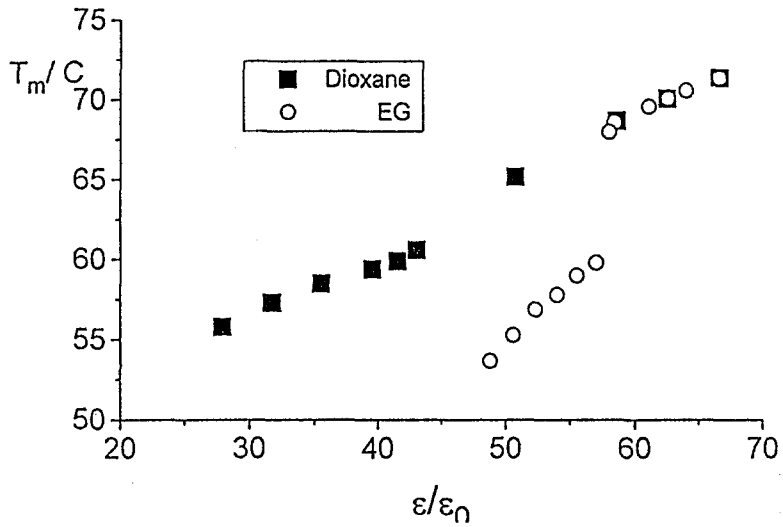

Fig. 1. Melting temperature of DNA solution in; (■) water/dioxane and (o) water/ethylene glycol mixtures versus the electric permittivity of solvent $\varepsilon/\varepsilon_0$.

2. Experimental

Calf-thymus DNA (Sigma, D 1501) was dissolved in 15 mM NaCl plus 1.5 mM sodium citrate (SSC 0.1 X) to make a stock solution of 4 mg of DNA in 10 ml. For the measurement, the DNA solution was brought to 20 μg/ml. All chemicals were of analytical degree and used without purification. Doubly distilled water of conductivity less then 1μS/cm, free from CO_2, and at neutral pH was used throughout. A Metrolab 2500 double beam spectrophotometer with a thermostating chamber was used to record the UV absorbance. Samples were placed in the spectrophotometer chamber at temperature below 10°C and then the temperature was slowly increased by circulating a water/glycerol mixture at controlled temperature. Temperature was kept at the desired value with the aid of a Lauda TUK 30D cryo-thermostat with a precision of 0.04°C. Sample temperature was measured with a Cole Palmer thermocouple thermometer 8110-15 with a precision of 0.1°C. Before proceeding to measure the melting temperature, the native state of each DNA sample was checked by recording its UV spectra between 230 and 320 nm at 25°C.

The electrical permittivity was recorded with a Hewlett Packard LF impedance analyzer 4192A at 20°C and 60°C.

3. Results and Discussion

Figure 1 shows the melting temperature plotted against the electrical permittivity for DNA in dioxane and ethylene glycol mixtures. The curves are almost identical for

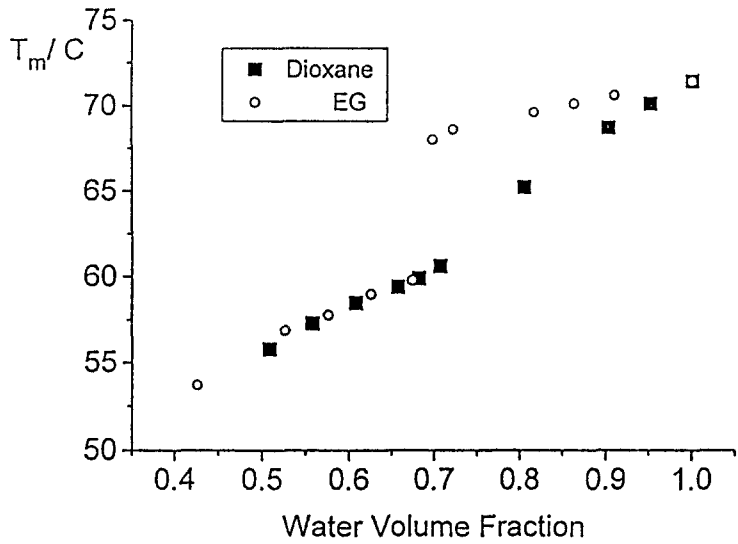

Fig. 2. Melting temperature of the same DNA solution shown in Fig. 1 plotted against the partial volume fracture of water in; (■) water/dioxine and (o) water/ethylene glycol mixtures.

both solutions to about $\varepsilon = 58$, in accordance with results of reference (3). For lower values of electrical permittivity the agreements vanish completely. Particularly for ethylene glycol, we observe an abrupt break in curve. For dioxane the discontinuity is not so dramatic and shows a linear realtionship down to $\varepsilon = 50$.

Looking at Figure 2 we see a coincidence of the melting temperature in the two solutions in a different range when it is plotted against the partial volume fraction of water. We see that at a 0.69 partial volume fraction of water a transition occurs and below that value both solutions exhibit the same behaviour.

Comparing the behaviour of the melting temperature as plotted in the two different ways, we can conclude that when the water content–measured as the partial volume fraction–remains over a critical value, the stability of the double helix depends on the charge screening. The dependence of stability on the charge screening is understood knowing that phosphate groups repelled each other. The ionic content and high electric permittivity produce a screening of such repulsion. On the other hand it seems that some minimum amount of water is required to effect stability. This fact has been mentioned already [4,5]. The results of reference [4] obtained by measurement of viscosity also gives a critical hydration value on the order of our observations.

It is striking that the critical hydration is obtained as volume fraction rather than water activity. Probably, some geometrical constraints related to the hydrophobic effect are the main factor to permit water to act as a structure-protection agent. The influence is so strong that the, otherwise important, effect of the charge screening is overriden. That water is essential to maintain biological structure is not a new fact, but the apparent fact that the effect can be evaluated by considering the occupied

volume and not the activity (or molar fraction), is an interesting aspect that warrants further investigation.

It is probable that the effect observed when water is under some critical quantity is due to a loss of the hydrophobic interaction between the DNA bases, which is known to be important [6]. This effect is added to the previously observed effect of destabilization by electrical interaction when permittivity is lower.

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References

1. Marmur, J., Round, R. and Shildrjraut, C.L.: Denaturation and renaturation of deoxyribonucleic acid, *Progress in Nucleic Acid Res. and Mol. Biol.: An Intl. Series*, Vol. 1, Davidson, J.N., and Cohn, W.E., eds., Academic Press, New York, 1963, pp. 231–249
2. Lazurkin, Y.S., Frank-Kametski, M.D., and Trifonov, E.N.: Melting of DNA: Its study and application as a research method, *Biopolymers* **9** (1970), 1253–1306.
3. de Xammar Oro, J.R., and Grigera, J.R.: Influence of the electrical permittivity and counter ions content of the media on the thermal denaturation of DNA, *Stud. Biophys.* **120** (1987), 51–57.
4. Frisman, E.V., Sloniisky, S.V., and Veselkov, A.N.: Influence of solvent structure on the conformation of the native DNA molecule, *Int. J. Quant. Chem.* **16** (1979), 847–855.
5. Eagland, D.: The importance of the water and hydration interactions in determining the conformational stability of biomacromolecules in aqueous solution, in: *Water, a Comprehensive Treatise*, Felix Franks, ed., Vol. 4, Plenum, New York, 1978, pp. 335–362.
6. Cupane, A., Vitrano, E., San Biago, P.L., Madonia, F., and Palma, U.: Thermal stability of poly (A) poly (U) complexes in H_2O and D_2O: isotropic effect on critical temperature and transition widths, *Nucleic Acid Res.* **8** (1980), 4283–4303.