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## What properties of non-electrolyte solutions does a DNA molecule feel?

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**Abstract:** As early as in 1953 it was reported that at low relative humidity of the atmosphere (about 70 %) DNA films were in A-form, while at higher humidity (more than 80%) they were in B-form. Relative humidity of the atmosphere corresponds to the water activity in the system. It was found in the beginning of the 70th that DNA transfers to the A-form when non-electrolytes, such as monoatomic alcohols, dioxane, tetrahydrofuran were added to water. It was reasonable to suppose that B to A transition of DNA occurs in the non-electrolyte solutions at the same values of water activity as in the moist atmosphere. This prediction was borne out. But in water solutions of very polar non-electrolytes such as methanol and ethylene glycol B to A DNA does not occur even at very low water activity values. It was supposed that A form of DNA can arise only in the medium with sufficiently low polarity. We used hyperfine splitting constant (A) of a nitroxide spin label determined from the EPR spectra as a measure of the liquid solvent polarity. It was found that DNA transition into B form occurs when A reached a certain value. Polarity of methanol and ethylene glycol is much higher than that of the solutions in which B to A DNA transition takes place. This transition also occurs in water solutions of trifluoroethanol. But in these solutions the transition takes place at hyperfine splitting values, which are much greater than in other non-electrolyte solutions. Water activity in the zone of B-A DNA transition in trifluoroethanol solutions does not correspond water activity which was calculated for other non-electrolyte solutions in which B-A transition is observed.

**Keywords:** water activity, non-electrolytes, A and B forms of DNA, polarity, EPR spectra, hyperfine splitting constants

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## 1. INTRODUCTION

In 1976 V.I. Ivanov gave a talk titled "DNA as a tool for the study of hydration of ions". It was done at the seminar of the Russian Academy of Sciences on the study of the structure of liquids and solutions headed by Prof. O.Ya. Samoilov. In this talk influence of the alkaline metal ions on the conformation of the double-stranded DNA molecule was discussed, in particular on the width of the narrow groove [1] (see also [2,3]). Influence of the ions on DNA was studied in water-methanol solutions in which DNA was in B family of conformations. Transition of DNA to A-conformation occurs in aqueous solution of dioxane, ethanol and isopropanol. As early as in 1953 Franklin and Gosling showed that DNA fibers transit into A-form when relative humidity became lower [4]. This was confirmed by Pilet and Brahm, who used spectral methods [5].

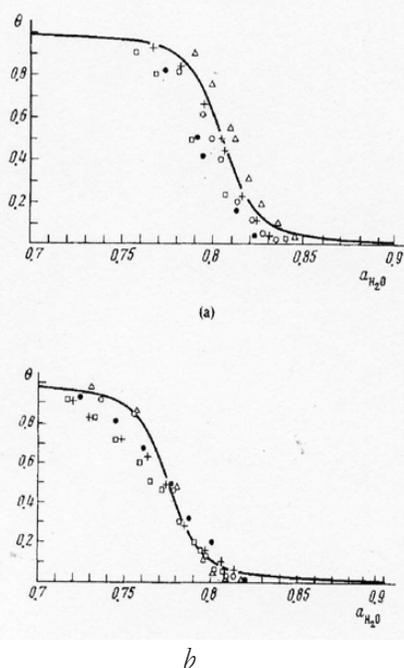
When experiments whose results were described in [1] were discussed, I put forward a supposition that transition of DNA to the A-form in aqueous non-electrolyte solutions (except containing methanol) occurs when water activity value was approximately 0.8, that is at the same water activity value as in moist atmosphere [4,5]. It can be recalled that water activity in the gaseous phase is equal to relative humidity:  $a_{\text{H}_2\text{O}} = P_{\text{H}_2\text{O}}/P_{\text{H}_2\text{O}}^0$ ;  $P_{\text{H}_2\text{O}}^0$  – pressure of the water vapour over pure water at the same temperature. Difference of the moist air and the ideal gas is not taken into consideration. For the

calculation of water activity in the solution it is necessary to know the composition of the vapour over this solution. When the calculations of water activities in the solutions in which B-A transition takes place were carried out, the supposition that B-A transition of DNA in these solutions occurs at same water activity values as in fibers and films in the moist atmosphere was verified. For the further check of this supposition a more thorough study of the B-A transition in the ethanol-water and isopropanol-water systems and in the triple systems isopropanol-ethanol-water, isopropanol-dioxane-water and isopropanol-tetrahydrofuran-water was performed [6]. In [6] method of water activity calculations is described and sources of data on the compositions of vapour phases over the solutions which are necessary for such calculations are given. In the paper [7] B-A transition in isopropanol-ethanol-water, isopropanol-dioxane-water systems is studied and described still more in detail and method of calculation of water activity is stated more thoroughly than in [6].

## 2. WATER ACTIVITY AND B-A TRANSITION OF DNA IN NON-ELECTROLYTE SOLUTIONS

Dependence of the fraction of the A form ( $\theta$ ) on water activity ( $a_{\text{H}_2\text{O}}$ ) in the systems: (a) water-ethanol-isopropanol; (b) water-ethanol-dioxane is shown in **Fig. 1**. Solid lines are theoretical curves, calculated using the following equation:

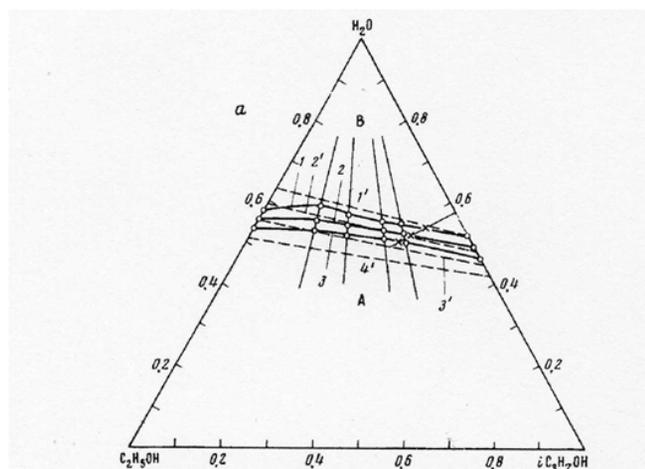
$$(1 - 2\theta/[\theta(1 - \theta)]^{1/2} = (1 - S)/(\sigma S)^{1/2}.$$



**Fig. 1.** B-A DNA transition in the systems: (a) water-ethanol-isopropanol; (b) water-ethanol-dioxane. Details are given in the text.

The equation was derived by Ivanov et al. in [8];  $\sigma$  is the cooperativity factor and  $S = \exp(a_{H_2O} - a_0Q)$ , where  $a_0$  is  $a_{H_2O}$  at the transition midpoint and  $Q$  is an adjustable parameter. Dots of different forms correspond to different content of the components.

The data for water-ethanol-isopropanol system are shown in **Fig. 2** in the form of



**Fig. 2.** Water-ethanol-isopropanol system. B-A transition  $\xi$  zone. Details in the text.

the triangle diagram. Concentrations are in mole fractions.

Solid lines are equal values of A-form fraction ( $\theta$ ); top to bottom – 0.25, 0.50, 0.75. Dash lines – lines of equal values of  $a_{H_2O}$ . From top to bottom: 0.82, 0.80, 0.78, 0.76.

### 3. POLARITY OF THE SOLUTION AND B-A TRANSITION OF DNA

It has been already mentioned that in water-methanol solution DNA is always in B family of conformations even at very low water activity values [1,9]. DNA retains B-conformation in almost anhydrous ethylene glycol [10,11]. The same can be said about DNA fibers containing inositol, in which DNA is in B-conformation in a practically dry atmosphere [12]

Thus, water is not the only substance that stabilizes the B DNA conformation. In the systems containing such substances as methanol and ethylene glycol water activity cannot be used as a parameter responsible for the B-A DNA transition.

It is intuitively clear that ethanol, isopropanol, dioxane and tetrahydrofuran are less polar substances than water, methanol and ethylene glycol. But how polarity of liquids and solution can be characterised?

The simplest and most physically lucid quantitative measure of polarity is the polarizability determined by the Clausius-Mosotti formula, derived in [13]

$$RT \ln \gamma_{H_2O} = \frac{\mu^2}{d^2} \left( \frac{\epsilon - 1}{2\epsilon + 2} - \frac{\epsilon^0 - 1}{2\epsilon^0 + 2} \right). \quad (1)$$

Here  $\gamma_{\text{H}_2\text{O}} = a_{\text{H}_2\text{O}}/x_{\text{H}_2\text{O}}$  – is water activity coefficient ( $x$  is mole fraction),  $\mu$  and  $d$  are dipole moment and molecular diameter of the most polar component (water),  $\epsilon^0$  is static dielectric of water,  $\epsilon$  is the same for the solution.

Thus, at least in some cases there exists a simple relation between water activity and polarity. Chemical potential of water  $\mu_{\text{H}_2\text{O}} = \mu_0 + RT \ln a_{\text{H}_2\text{O}} = \mu_0 + RT \ln \gamma_{\text{H}_2\text{O}} + RT \ln x_{\text{H}_2\text{O}}$ , where  $a_{\text{H}_2\text{O}}$  – water activity,  $\gamma_{\text{H}_2\text{O}}$  – is water activity coefficient,  $x_{\text{H}_2\text{O}}$  – is mole fraction of water in the system. Combining with c (1), we can write

$$\mu_{\text{H}_2\text{O}} = \mu_0 + \frac{\mu^2}{d^2} \left( \frac{\epsilon - 1}{2\epsilon + 2} + \frac{\epsilon^0 - 1}{2\epsilon^0 + 2} \right).$$

However, we did not calculate polarity of our solutions. We used an empiric method of measuring liquid solution polarity with the help of the hyperfine splitting constant (A) of a nitroxide spin label in the EPR spectrum [14]. Value of the hyperfine splitting constant (A) depends on the interaction of the unpaired electron the N-O group with the surrounding medium.

The details see in our publication [15] and particularly in [16].

The more polar is the medium, the greater is hyperfine splitting constant A, we used 2,2,6,6-tetramethyl-1N-oxypiperidinol and of 2,2,6,6-tetramethyl-1N-oxypiperidine acetic acid as the spin labels. We shall use the data only for the first label in this paper. We dissolved the spin labels in the non-electrolyte solutions of the compositions in which, according to our circular dichroism studies, 50%

of DNA was in A-form and 50% was in B-form. EPR spectra were registered on a Varian E-104 spectrometer. Value of A was measured between the central and low-field components of the triplet spectrum (in Gauss, G).

Data for midpoints of B-A DNA transition in the systems water-ethanol-isopropanol and water-ethanol-dioxane are in the given **Table 1** – composition of the solutions, water activity and polarity indices A and  $\Delta A$  in Gauss (for 2,2,6,6-tetramethyl-1N-oxyl piperidinol).  $\Delta A = A - A_0$ .  $A_0$  – value of A in the absence of the electric field.

Hydrogen bond contribution to  $\Delta A$  was also determined by us [15,16], but this question will not be discussed in the present paper.

According to EPR criterion polarity of aqueous methanol solutions is beyond the limits of polarity values in which DNA exists in the A-form. The same can be said about water-ethylene glycol solutions. In anhydrous methanol as well as in other monoatomic alcohols

**Table 1**  
Properties of the solutions in which half of DNA transforms from form B into form A ( $\theta = 0.5$ ) at 25°C.

System	$X_1$	$X_2$	$X_3$	$a_{\text{H}_2\text{O}}$	A,G	$\Delta A,G$
Water-ethanol-isopropanol	0.556	0.444	0	0.786	16.80	1.50
	0.536	0.332	0.132	0.787	16.80	1.50
	0.527	0.264	0.209	0.791	16.76	1.45
	0.526	0.183	0.291	0.801	16.75	1.45
	0.523	0.141	0.336	0.805	16.75	1.45
Water-ethanol-dioxane	0.498	0	0.502	0.811	16.70	1.40
	0.576	0.372	0.052	0.811	16.80	1.50
	0.592	0.329	0.079	0.780	16.80	1.50
	0.529	0.216	0.055	0.774	16.80	1.50
	0.645	0.145	0.209	0.772	17.75	1.45
	0.701	0	0.299	0.770	16.75	1.45

DNA is not stable and precipitates. In almost anhydrous ethylene glycol double stranded DNA is quite stable and, according to circular dichroism spectra, is in B-form. It was shown later that in ethylene glycol DNA denatures at lower temperature than in water [18,19]. It would be interesting to realize the B-A DNA transition in the solutions ethylene glycol – less polar non-electrolyte (e.g. ethanol or isopropanol) and to determine corresponding EPR polarity values of such solutions. It was not our fault that we did not manage to fulfil this project.

#### 4. ENTHALPY OF B-A DNA TRANSITION

Among other thermodynamic properties of DNA in aqueous non-electrolyte solutions we managed to determine enthalpy B-A transition in the water-ethanol system [20,21]. DNA B-A transition occurs in interval 36.4 – 52.2 mole % of ethanol in this system (at room temperature). The heat effect connected with the change of the concentration of the solution is very great and it is practically impossible to measure on its background a small change of enthalpy caused by the B-A transition itself. It is known that guanidinium cation  $C(NH_2)_3^+$  stabilizes B-form of DNA at the concentration of less polar component which is higher than B-A transition interval. In order to determine the enthalpies of B-A transition enthalpies of the interaction of the guanidinium chloride solutions with DNA in

B- and A-conformations were measured calorimetrically in the broad interval of ethanol concentrations in aqueous solution containing DNA at 298.15 K. Concentrations of sodium ions were measured in these DNA and guanidinium containing water-ethanol solutions were measured potentiometrically with the help of sodium sensitive electrode. It was found that interaction guanidinium chloride interaction with DNA leads to complete replacement of sodium ions, initially bound to DNA, by guanidinium ions.

Enthalpies of dissolution of guanidinium chloride in water-ethanol solutions in the interval of concentrations (from pure water up to 90 mole % of ethanol) were also measured.

The formula  $\Delta H_{\text{exp}} = \Delta H_1 + \theta \Delta H_2$  was used for the calculation of the enthalpy of the B-A transition ( $\Delta H_{\text{exp}}$  is experimental value of the enthalpy change after adding the guanidinium chloride to DNA containing water ethanol solution,  $\Delta H_1$  is enthalpy of substitution of sodium ions by guanidinium ions,  $\theta$  is DNA A-form fraction in the solution of the given composition,  $\Delta H_2$  is enthalpy of B-A DNA transition). It turned out that  $\Delta H_1$  and  $\Delta H_2$  values slightly depend on the solution composition. It was found that  $\Delta H_1 = 0.93 \pm 0.04$ ,  $\Delta H_2 = 2.50 \pm 0.10$  J/mole. Thus enthalpy of the B-A transition is small, which is in agreement with the fact that B-A equilibrium is little affected by temperature change [22].

### 3. CONCLUSION

Soon after the publication of the paper [16] the group of V.I. Ivanov began to study B-A DNA transition in water solutions of trifluoroethanol  $\text{CF}_3\text{CH}_2\text{OH}$ . It was found that DNA did not precipitate in these solutions even at very high non-electrolyte concentration, unlike used earlier ethanol, isopropanol and other substances whose polarity was less than that of water. We obtained EPR spectra of the spin label in trifluoroethanol and its water solutions and found that  $A$  and  $\Delta A$  values in trifluoroethanol were almost the same as in water and they practically did not depend on water content in the solution. In those days I could not find experimental data about composition of vapour above these solutions, and it was not possible to calculate water activities in this system. But it was clear that in trifluoroethanol solutions DNA respond not to the same properties which determine  $A$  and  $\Delta A$  values in other solutions.

In the beginning of the 2000th we were working on the paper dedicated on memory of L.E. Minchenkova [23]. In this paper B-A transitions of polynucleotides trifluoroethanol solutions were discussed. By that time the data about vapour composition above trifluoroethanol solutions had appeared already and it became possible to water activity in D-A transition field. It turned out that in trifluoroethanol solutions B-A transition occurred at higher water activity values

than we had observed in the solutions containing ethanol, isopropanol, dioxane and tetrahydrofuran. The data for B-A transition in trifluoroethanol solutions for high molecular DNA obtained in 70th and beginning of 80th also testified that B-A transition occurred not in the interval of water activity values as it was observed in other binary water-non-electrolyte systems. To which property of trifluoroethanol solutions DNA reacts when A-form of DNA arises is not clear up to now. Their polarity is close to polarity of water and water activity is too high for the possibility of DNA to exist in the A-form

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