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EXTERNAL MAGNETIC FIELD EFFECT ON VISCOMETER EFFLUX TIMES OF CALF THYMUS DNA IN 0.0172 M KCl SOLUTION DURING THE THERMAL DENATURATION PROCESS

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ABSTRACT

Experiments show that during the thermal DNA denaturation process an applied magnetic field of 10 kG increases the shear viscosity (measured efflux time) of calf thymus DNA in 0.0172 M KCl solution over the viscosity of the same solution at the ambient earth field (or "no" field) condition. There is also observed an increase (although slight, about 0.1°C) in the thermal denaturation temperature of the process.

INTRODUCTION

Current literature shows [1—5] that an externally applied magnetic field influences the behavior of various biological systems, such as organs, cells, subcellular and molecular structures. It has also been shown that an applied magnetic field affects weakly the transport properties of water and aqueous electrolyte solutions [6—19] as well as various structural parameters of liquid crystals and polymeric organic substances [20—24]. On the other hand, there have been recent studies to measure magnetic fields produced by biological current sources, such as human heart, brain and skeletal muscle [25—31]. In this work, however, we have studied the behavior of the calf thymus DNA denaturation process [32—36] through determining the applied magnetic field effect on the efflux time of DNA in 0.0172 M KCl aqueous solution during the denaturation process. As a result of a series of measurements performed under carefully controlled experimental conditions, it was shown that the applied magnetic field of 10 kG increases the efflux time of calf thymus DNA in 0.0172 M KCl aqueous solution (Figs. 3 and 4). There was also indication that the thermal denaturation temperature, T_m, was increased slightly, from 67.2°C to 67.3°C.

EXPERIMENTAL

Apparatus

Details of the apparatus (Fig. 1a, b) and methods used for the determination of measured calf thymus DNA aqueous solution outflow times are given

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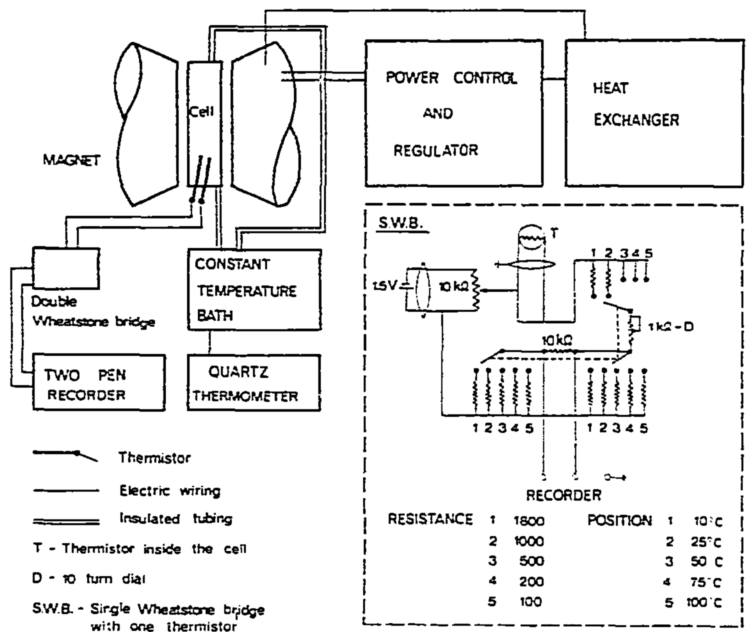

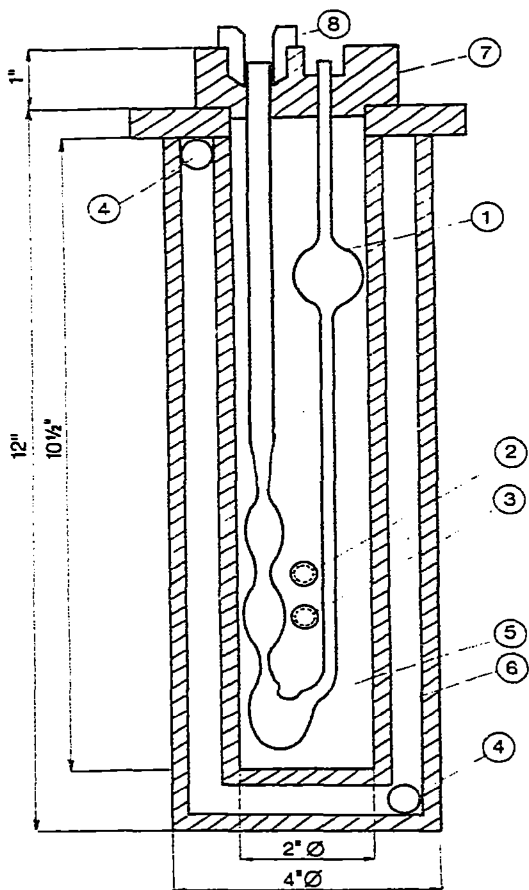

Fig. 1. (a) Apparatus. (b) The viscometer cell. 1, Cannon—Fenske opaque viscometer; 2, thermistor inside transformer oil; 3, thermistor inside the water jacket; 4, 0.5 in. diam. tap for cooling water inlet and outlet; 5, bath with transformer oil; 6, bath with circulating water; 7, holder for the viscometer; 8, screw tightening the viscometer.

by Lielmezs et al. [14] and Lielmezs and Aieman [10,11,13] in their studies of the weak transverse magnetic field effect on shear viscosities of water and a series of aqueous electrolyte solutions. The apparatus consists of two parts: the Varian Associates 12-in. low impedance electromagnet (Model V-7300) and the viscometer—temperature bath assembly. The flow diagram of the apparatus is shown in Fig. 1. The electromagnet system is equipped with field regulated Varian Associates magnet power supply (Model V-7800) and a heat exchanger. The homogeneity of the magnetic field for a pole cap of 7-in. diameter and a 4-in. air gap is 700 mG over 1/2-in. diameter measured at 13.5 kG magnetic field (Fig. 2). The viscometer—temperature bath assembly consists of a viscometer cell (Fig. 1a, b), a constant temperature bath control (Fig. 1a), and a measuring—recording system to record and control by means of thermistors the viscometer and bath temperatures continuously (Fig. 1a, b). The operation of this assembly is described in detail by Lielmezs et al. [14]. The Cannon—Fenske opaque (calibrated, reverse-flow, No. 100 A 737) viscometer was modified by shortening it to 7 in. total length (then recalibrated) to match the diameter (7 in.) of the magnet pole (Fig. 2). During the measurement time the viscometer was immersed in a constant temperature transformer oil bath (temperature measured and controlled by thermistors to ±0.01°C accuracy) (Fig. 1b, inner shell). The rate of the calf thymus DNA aqueous solution flow in the viscometer was measured with a stopwatch (accuracy better than ±0.02 sec).

Procedure

Samples

Miles Laboratories (PTY) Ltd., Elkhart, Indiana, U.S.A., supplied the calf

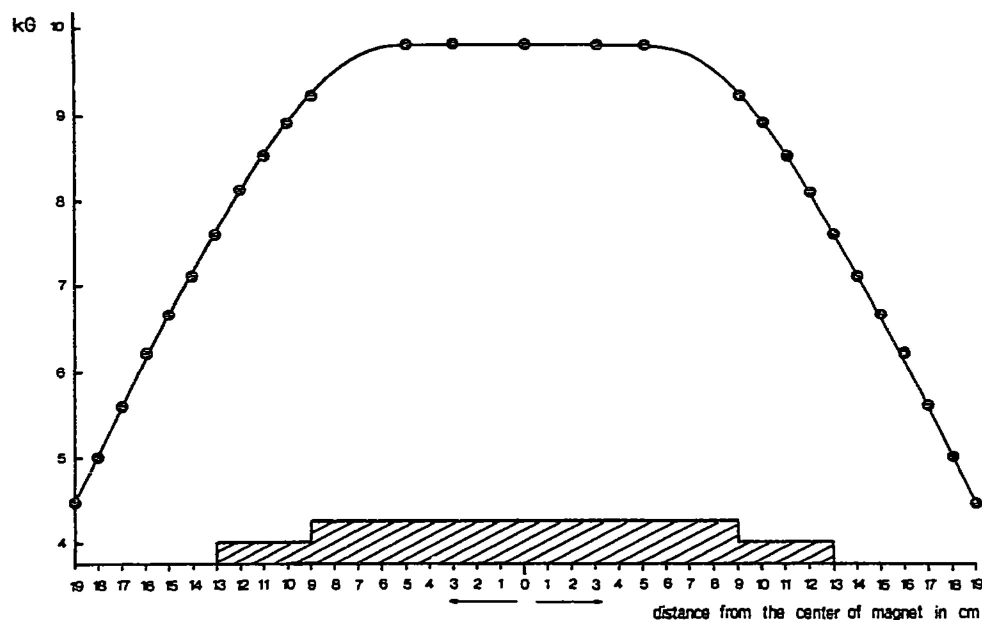

Fig. 2. The homogeneity of the applied field across the magnet pole gap.

thymus DNA sample (Batch 7038, Code 36-155) in the form of a freeze dried, salt free, pure white preparation, soluble in water and dilute buffers. Assayed material contained moisture (1.1%), protein (0.4%) and RNA (5%). The purity of the sample was 89.2%, and it is thermostable.

The batch viscometer sample was prepared by dissolving the supplied calf thymus DNA to 0.056% concentration in 0.0172 M KCl aqueous solution at pH = 6.7 ± 0.3. This solution was kept in refrigerated storage at +10°C. The same batch was used for all efflux time measurements.

Efflux time measurements

During the thermal denaturation process of DNA in 0.0172 M KCl aqueous solution, all efflux time measurements were performed at the given earth magnetic field (the reference state, or "no" field condition) and under the applied magnetic field of 10 kG. Both sets of measurements were then compared (Figs. 3 and 4).

To perform the efflux time measurements, the viscometer was placed symmetrically in the 4-in. air gap between the magnet pole faces (Fig. 1a), so that the magnetic field force lines, when applied, cut transversally the flowing DNA solution in the viscometer.

To perform the efflux time measurements at the given earth magnetic field, or at the reference state (the "no" field condition), the calf thymus DNA solution was removed from the refrigerator, preheated for 30 min, brought up to 61.75°C, then placed in the viscometer (already housed in the

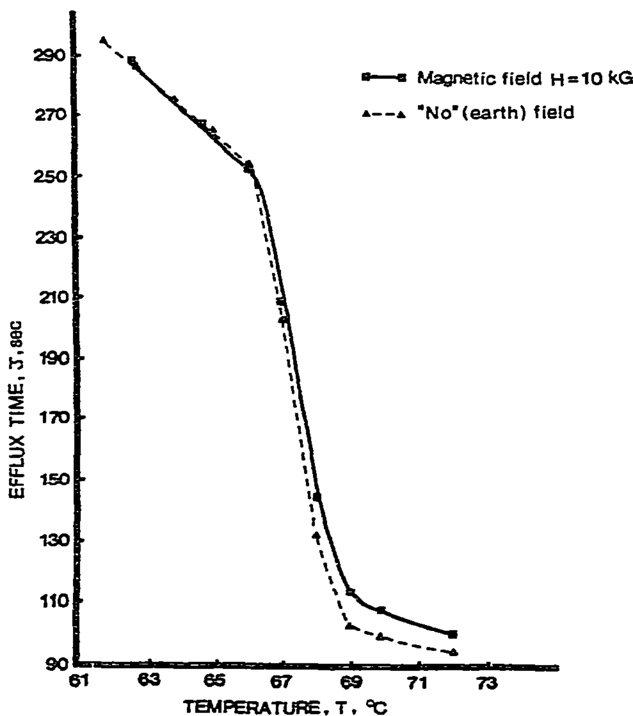

Fig. 3. Efflux time, τ, vs. temperature, T, comparing measurements made at an applied magnetic field, H, of 10 kG and "no" field condition.

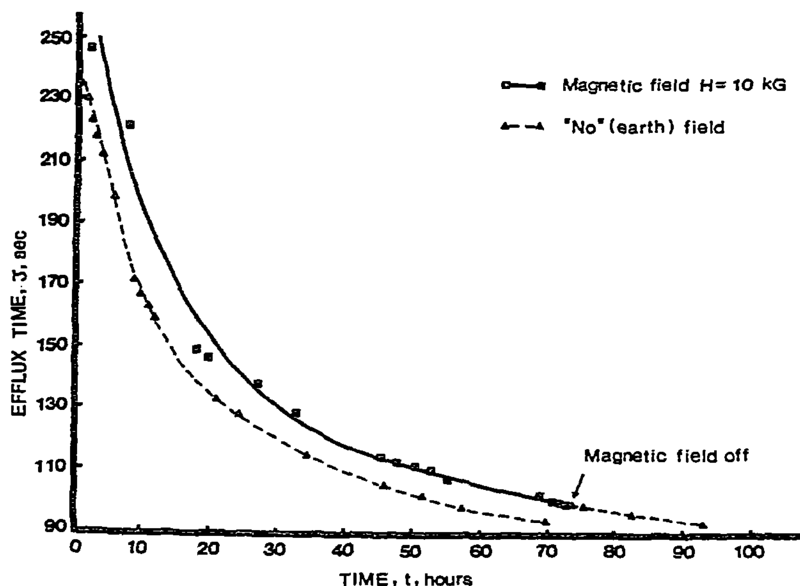

Fig. 4. Efflux time, τ, vs. heating time, t, at a constant temperature of 72.03°C comparing measurements made at an applied magnetic field, H, of 10 kG and "no" field condition.

viscometer cell, Fig. 1b) and kept there, at this temperature for a further 17 h, at which time the solution had reached stable condition. At this point the final (stable) efflux time was measured for the initial 61.75°C isotherm. Efflux times for the other "no" field or reference state isotherms (62.72, 63.85, 65.00, 66.00, 66.94, 67.92, 68.90, 69.90 and separately at 72.03°C) were measured in a similar fashion.

To measure the efflux times of the given DNA aqueous solution under the influence of the applied magnetic field (H = 10 kG), the following procedure was used. First, a new sample was taken from the common refrigerated solution stock, placed in the viscometer and kept for 22 h at 61.62°C and at "no" field condition to assure that the solution in the viscometer had reached the thermally stable condition. The efflux times were then measured at "no" field condition. Upon accounting for the temperature difference of 0.13°C between the two isotherms (61.75 and 61.62°C), it was found that the measured efflux times were the same. Having thus assured ourselves that the originally prepared DNA in 0.0172 M KCl solution had not lost the initially observed structural characteristics while stored, we could proceed to measure the solution efflux time under the influence of the applied magnetic field. To do this, the viscometer containing the preheated DNA in 0.0172 M KCl solution (Fig. 1a, b) was subjected to the continuously applied magnetic field of strength H = 10 kG. The subsequent efflux time measurements describing the DNA denaturation process were performed at the following isotherms: 62.63, 64.66, 66.00, 66.90, 67.94, 68.90, 69.80 and separately at 72.03°C (Figs. 3 and 4).

RESULTS

The results of this work are summarized in Figs. 3—5. which show the applied magnetic field effect (applied field strength $H = 10$ kG) on the efflux time of calf thymus DNA in 0.0172 M KCl solution during the thermal denaturation process. Figure 3 shows the DNA solution efflux time τ (sec) sigmoidal type dependency on temperature T(°C) by comparing measurements obtained at the so-called "no" field (or the ambient earth field H_0 with the associated efflux time τ_0) condition with the measurements obtained under the influence of the applied magnetic field (applied field strength $H = 10$ kG, associated efflux time τ_H). As seen from Fig. 3, the applied magnetic field H appears to become notably effective only during the denaturation process by increasing the efflux time at the given temperature ($\tau_H > \tau_0$, T fixed). Furthermore, it appears that the applied magnetic field for the given solution has slightly increased the observed denaturation temperature, T_m, from 67.2°C ("no" field condition) to 67.3°C (applied field $H = 10$ kG). Figure 4 shows the effect of the applied magnetic field H on the efflux time τ if the DNA solution is heated at constant temperature $T = 72.03$°C (taken below the observed melting temperature T_m, Fig. 3). Again we see (Fig. 4) that at a given heating time t(h) on the 72.03°C isotherm, $\tau_H > \tau_0$. Figure 4 also shows that the largest magnetic field effect is found at the beginning of the isothermal denaturation process *, for instance, efflux time τ_H has increased by 17.3% at 5 h of heating under the influence of the applied magnetic field, while at 70 h heating time of solution at constant temperature at magnetic field strength 10 kG, the corresponding τ_H increase is only 6.4%. If we define $\Delta\tau = \tau_H - \tau_0$, and plot this efflux time difference against the corresponding heating time t values (Fig. 5), we see that at large heating times, keeping other parameters fixed, $\Delta\tau$ decreases. This re-enforces the already made observation that the applied magnetic field of the given strength of $H = 10$ kG (over that of the ambient earth magnetic field) within the presented measurement accuracy, influences the DNA solution only during the denaturation process. Whether this remains true at higher applied magnetic field strength, is a question of further experimental work.

DISCUSSION

The obtained results (Figs. 3—5) show that the applied magnetic field of strength $H = 10$ kG (over that of the ambient earth magnetic field) possibly weakly (still additional evidence needed) affects the efflux time of the given DNA solution before and after the denaturation, while, on the other hand, very strongly increases the efflux time of DNA solution (at some isotherms more than 17%) during the denaturation process (Figs. 3—5). It appears therefore that through the increase of the efflux time of solution, the applied magnetic field has in effect retarded the course of the DNA denaturation process. Whether this means that the applied magnetic field has stabil-

* At the start of heating of the given solution, a thermal adjustment process appears to occur (see Figs. 4 and 5).

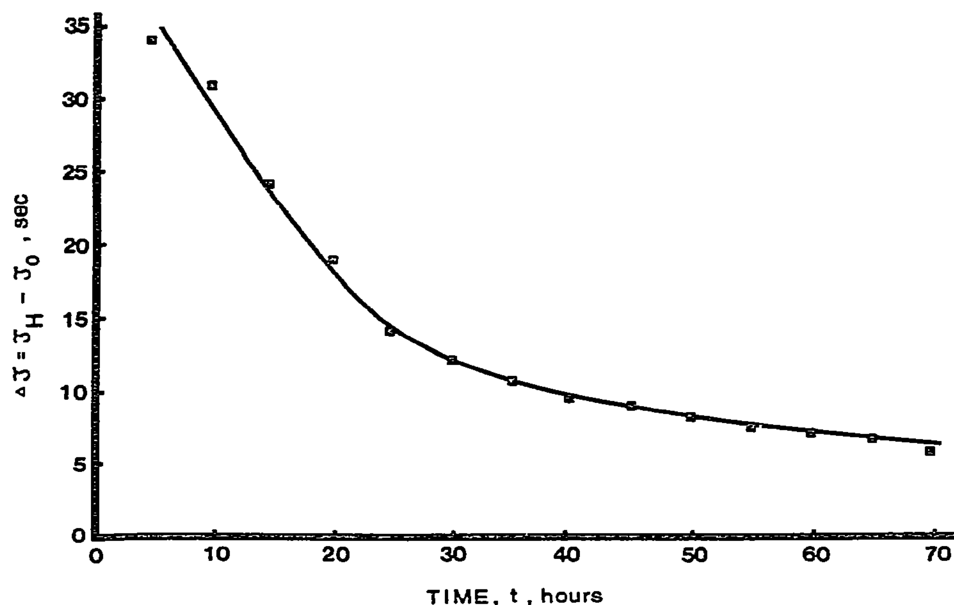

Fig. 5. Efflux time difference, $\Delta\tau = \tau_H - \tau_0$, vs. the corresponding heating time, t.

ized the DNA structure in solutic, or for that matter retarded the double helix-coil unwinding, separately or in combination with various environmental and solute—solvent interaction modes [37—46] during the denaturation process, is an open and very complex question. Previous studies [10,14, 16] show, however, that an applied magnetic field changes, although weakly, the shear viscosity of pure water and of KCl—H_2O solution. Thus an external magnetic field of strength H = 10 kG at T = 25°C increased the magnitude of viscosity of pure water by only 0.25% while the viscosity of 0.01 M KCl—H_2O solution at H = 12 kG and T = 25°C increased it by 0.?42%. These weak magnetic field effects on viscosities of diamagnetic liquids [10,14,16] are of the same magnitude as the yet uncertain observed viscosity changes in the magnetic field of the given diamagnetic DNA aqueous solution before and after the denaturation process. The fact that an applied magnetic field seems to affect only very weakly the viscosity and possibly the structure [10,14, 16] of a diamagnetic aqueous solution is further strengthened by a study [47] of the applied magnetic field effect on the degree of orientation of the calf thymus DNA molecule in an aqueous solution of 0.15 M NaCl and 0.015 M Na-citrate buffer, pH = 7.0. Measurements showed that the calf thymus DNA in the given solution at a magnetic field of 120 kG was oriented only by 0.88%. However, as this DNA solution approached its melting temperature T_m, the magnetic orientation vanished.

These observations seem to directly support the assertion that the observed large efflux time increases of DNA aqueous solution (Figs. 3—5) are interpretable through the possible retarding action of cooperatively occurring DNA structural changes and the associated physicochemical phenomena in solution during the denaturation process.

We assume that the given calf thymus DNA sample can be characterized by the classical Watson—Crick model [48,49]. That is, the DNA structure in its native state consists of a double stranded helix of two polynucleotide chains wound around each other and held together by interactions between layers of the purine (adenine or guanine) and pyramidine (cytosine or thymine) bases and twisting 36° for every pair of bases in such a way that adenine is hydrogen bonded to thymine (two hydrogen bonds) and guanine to cytosine (three hydrogen bonds). The stability of this structure [38—46, 50,51], especially in aqueous solution, is increased by base stacking, hydrophobicity (series of intra- and intermolecular interactions, mainly of electrostatic, induction, dispersion and charge transfer type), solvent and conformational adjustment effects.

We also assume that the denaturation * process of the given calf thymus DNA in solution can be described via the cooperative collapse of the secondary structure of the DNA double helix involving the separation (or unwinding) of the two chains of the double helix and their subsequent transformation into random coils and possibly other units and subunits upon heating at fixed ionic strength and pH value. We note that by introducing an applied external magnetic field over that of the ambient earth magnetic field, we have changed the DNA solution magnetic state from H_0 (ambient reference earth magnetic field state) to H (applied external magnetic field state) and then, keeping this new magnetic state H fixed, we have subjected the DNA solution by heating (that is, going from isotherm to isotherm, stepwise, we have changed correspondingly the thermal state by increasing the thermal energy of molecular motion, RT, of the given solution) to undergo the thermal denaturation process. It appears that if the applied magnetic field H is kept fixed for all thermal state, RT, changes for the given DNA solution, then, with respect to the constant (kept fixed) H value, temperature T (thermal state property) would become the perturbing parameter. Figures 4 and 5 (already discussed) point this out, indicating that the isothermal denaturation ($T = 72.03°C$), by giving comparatively the measured magnetic field effect decrease in time, may well be viewed as a relaxation process. It is of interest to note that at the end of isothermal denaturation the sudden removal of the applied magnetic field H seemingly did not alter the course of the process (Fig. 4).

To express the denaturation as a thermally induced cooperative process [55], we assume that the breaking of one bond facilitates the bond breaking of its neighbors; that is, we have the direct breaking of bonds caused by the specific changes in the molecular environment, and the indirect breaking of bonds associated with the break-up of the nearest neighbors. In effect we have an interdependence of states between various links of the given molecular structure; i.e. any link state depends on the states of its neighbors. Yet this interdependence occurs in such a manner that at the completion of the denaturation process (at the given reference magnetic state, the ambient

* In view of more current thermal subtransition theories, the thermal denaturation of native DNA in moderately dilute salt solutions may take place as a summation of discrete steps, each of which is a component part of the total transition [52—54].

earth magnetic field, H_0), the two chains of the DNA double helix in solution have separated into random coils. The results indicate (Figs. 3—5) that the applied magnetic field H ($H > H_0$) changes the nature and degree of cooperativity of the transition by increasing the stability (i.e. increased retardation) of the denaturation process, and at the same time biasing the previously found thermally induced random nature of the separation of coils. That is, the introduced magnetic state change (state $H_0 \rightarrow$ state H) affects the thermally denaturating system (DNA solution at thermal state RT) by interdependently influencing the DNA-structure stabilizing molecular interaction modes such as the already noted hydrogen bonding, base stacking, hydrophobicity, solvent and conformational adjustments. Additionally, there may be interactions associated with magnetic field inhomogeneities as caused by possible transition metal inclusions in DNA [56]. The magnitudes of these native state stabilizing intermolecular interaction energies between various DNA components (A—T, G—C) are large; for instance, the hydrogen bonding contributes of the order of 5—15 kcal mole^{-1} per base pair [38—46]. This is the total hydrogen bond energy for the given base pair, and it may be decomposed into individual base pair hydrogen bond energy component contributions. For instance, Egan et al. [46] specify that the Watson—Crick DNA model A—T base pair has the following hydrogen bond energy components

$E_{\text{electrostatic}} = -8.99$ kcal mole^{-1}; $E_{\text{polarization}} = -1.09$ kcal mole^{-1};
$E_{\text{dispersion}} = -1.06$ kcal mole^{-1}; $E_{\text{repulsive, short range}} = +3.81$ kcal mole^{-1};
$E_{\text{total hydrogen bond, A—T pair}} = -8.04$ kcal mole^{-1}

There are other possible hydrogen bond energy distributions; for instance, a given base pair may possess several configurations each of which has a different stability, i.e. different hydrogen bond strength [38—46].

Of specific interest are the overlapping energy arrangements (or base stacking) of the planar, conjugated aromatic (purine, pyrimidine) molecules placed in the double helix at Van der Waals distances of separation [38—46, 50,51]. These base stacking interactions (inductive, electrostatic, dispersion forces) strongly influence the secondary structure and the overall stability of the DNA molecule. While the stacking interactions may be expressed as inductive (or polarization), electrostatic and dispersive forces, their resulting action is determined through an interplay of geometrically permitted degrees of freedom (such as the base pair relative rotation about the helix axis, the vertical and horizontal translations), and a certain composition variability found in the secondary structure of the DNA molecule. Altogether these interactions approximately may contribute as much as the hydrogen bonding.

The stability of the double helix is further modified by the environment, i.e., first by the solvent effect; for instance it is found [39,43,45,46,50] that the G—C pair formation is destabilized while the A—T pair formation is mildly enhanced * in water. Then, additionally, stability modifications may

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* It is of interest to recall that a G—C pair is thermodynamically more stable than an A—T pair; i.e. more energy is needed to denature a G—C pair than an A—T pair [57,64—66]. Yet this stabilizing energy difference may be apparently decreased in solution.

be caused by the existence of a strain in the backbone, associated with the conformational adjustment energies [39,43,45,46]. Also, it should be recalled that in solution the electrostatic repulsion between phosphates on the negatively charged phosphate lattice of the DNA molecule destabilizes the helical structure, while the charge neutralization on phosphates by cations (i.e. K^+ ion) stabilizes the double helix [57—63] *.

For a quasistatic reversible thermodynamic process (or state change), the energy of interaction ΔE of a substance of molar susceptibility χ_M placed in a magnetic field of strength H_1 may, in general, be expressed as

$$\Delta E = -1/2 \chi_M H^2 \quad (1)$$

The existing diamagnetic anisotropy associated with the stacked coplanar arrangement (as produced by the interactions between the π-electrons of the base pairs) of the purine and pyrimidine rings is known. As a matter of fact, the diamagnetic anisotropy of the base pairs $\chi_{/\!/} - \chi_{\perp}$ has been calculated [67, 68] to exceed that of benzene ($\chi_{/\!/} - \chi_{\perp \text{benzene}} \approx 59.7 \times 10^{-6}$ e.m.u.) at room temperature by a factor of 1.098 and 0.827 for A—T and G—C, respectively. If additionally at room temperature the calf thymus DNA molecule contains [69] 42% G—C and 58% A—T, and if the applied magnetic field strength H is 10 000 G, then the available anisotropic diamagnetic interaction energy ΔE may be estimated as [eqn. (1)]: $\Delta E \approx 28.09 \times 10^2$ erg mole^{-1} = 67.14×10^{-6} cal mole^{-1} = 28.09×10^{-5} J mole^{-1}. The thermal energy RT at 25°C, however, may be estimated as 2.479×10^{10} erg mole^{-1} = 5.92×10^2 cal mole^{-1} = 2.479×10^3 J mole^{-1}. It is of interest to note that small changes in the DNA molecular conformation caused by the bending and stretching of covalent bonds may affect effectively local structural strains as well as the steric intra- and inter-molecular interactions.

To estimate the possible magnitude of this type of interaction, assume that the involved potential energy ϵ for the corresponding bending and stretching modes may be expressed in terms of small deviations from the stable equilibrium position of the given bond distances and angles through the Hookes law as a first approximation **. Then, first for the bond stretching, we may write

$$\epsilon(X) = 1/2\, k_x\, (\Delta X)^2 \quad (2)$$

where $\Delta X = X - X_0$, is the displacement from the given stable equilibrium position, X_0 being the equilibrium bond distance, while k_x is the needed bond stretching force constant.

* Principal stabilization of the helix in solution is provided by the ionic bonding between K^+ ion and the anionic phosphate group. The Cl^- ion has lesser effect on the thermal stability of the DNA molecule because the ionic interaction is reduced as the inverse of the distance between two ions; i.e. the larger distance is found between Cl^- and phosphate ions, but the smaller between K^+ and phosphate.

** Compare this with the work by Lielmezs et al. [14] in which they used Pople's [70] hydrogen bond bending model to estimate the possible deformation in the hydrogen bond distortion angle as caused by an applied magnetic field of strength H = 10 kG.

Similarly, for the bond bending we write

$$\epsilon(\theta) = 1/2\, k_\theta\, (\Delta\theta)^2 \quad (3)$$

Where $\Delta\theta = \theta - \theta_0$ is the angular displacement from the stable equilibrium position. θ_0 is the equilibrium bond angle, while k_θ is the corresponding bond bending force constant.

Equations (2) and (3) may be used to indicate the possible applied magnetic field effect on the conformational behavior, for instance of the DNA-A form molecule. To do this, we have selected the following structural data: stretching force constants [71], k_x, given as $k_{C-C} = 25.33 \times 10^4$ kJ mole^{-1} nm^{-2}; $k_{C-N} = 29.33 \times 10^4$ kJ mole^{-1} nm^{-2}; $k_{C-H} = 28.31 \times 10^4$ kJ mole^{-1} nm^{-2}; bending force constant [71] k_θ given as $k_{C-C-C} = 0.217$ kJ deg^{-2} mole^{-1}; corresponding bond lengths given as [72]: $X_{C-C} = 1.53$ Å; $X_{C-N} = 1.32$ Å; $X_{C-H} = 1.07$ Å; and one of the favorable rotational angles ψ' (Sundaralingam's [73] notation) around the C_3'—C_4' bond in the DNA-A conformational form, $\psi' = 76°$. It should also be noted that just this angle affects the displacement of the C_3' and C_2' atoms of ribose from the plane.

If we assume that the estimated magnetic interaction energy $\Delta E = 28.09 \times 10^{-5}$ J mole [eqn. (1)] is equal to the involved potential energy ϵ for the corresponding bending and stretching modes, then, using eqns. (2) and (3) and the given data, we find that for this interaction energy, first, the bond stretching displacement from the given stable equilibrium position will range from 0.0097 to 0.0104% and to 0.0132%, while for the C_3'—C_4' bond bending angle $\psi' = 76°$, it will be 0.0022% or 5.7 angular seconds.Although this percentage deformation, both the bond length and the angular, is small, nevertheless the structure of the backbone of the DNA double helix has been distorted. To what extent these backbone distortions may affect the overall stability and the secondary structure of the DNA double helix as well as the various helix—environment interaction modes of the given aqueous system, is an open question. However, the aromatic, i.e. strongly conjugated $\sigma\pi$-bonds found in the basic aromatic ring compounds (purine and pyrimidine) of the DNA double helix structure, are characterized by the fact that their π-electron clouds are to some extent shared by all the atoms of the entire conjugated ring [74,75]. The π-electrons thus flow more or less freely around the perimeter of the aromatic rings yielding the so-called ring currents [74]. This nearly free π-electron flow is the principal cause of the large anisotropy of the magnetic properties including magnetic susceptibility found in the aromatic rings [74,75].

In general, the magnitude of the diamagnetism of the conjugated base ring system will depend on the π-electron density (i.e., the area of orbit over which the π-electrons are distributed and on the π-electron number). However, the nature of the π-electron density distribution suggests that the magnetic susceptibility of the conjugated base rings should include both the diamagnetic and to some extent the paramagnetic components [67,68,74]. Even if for the aromatic base rings the anisotropies of the diamagnetic susceptibility are large [74], possible distortion of the backbone of DNA helix due to the applied magnetic field may increase further the magnetic inhomogeneities in both the DNA structure and the interacting surrounding aqueous solution.

It should be recalled that another magnetic inhomogeneity increase may come through the presence of the already noted ferromagnetic inclusions in DNA samples [56]. During the denaturation process of the DNA molecule the stacking interactions between the adjacent conjugated bases will dissolve. Accordingly, for these adjacent aromatic ring bases the π-complex overlap electron density will decrease, i.e. orbitals confining π-electrons and the number of π-electrons in each of these orbitals will decrease. Hence, by decreasing the magnitude of diamagnetism we will have increased the apparent paramagnetism in the DNA and aqueous solution system.

It is possible that under the influence of the given applied magnetic field, the cooperatively interacting magnetic moments, as modified by the feasible structural distortions of the DNA molecule, will be of sufficient strength to slow down the cooperative denaturation process of the DNA double helix in solution (see the previous discussion, Figs. 3—5).

An estimate of the possible extent of the applied magnetic field effect on this cooperative collapse of the secondary structure of the DNA double helix is provided by the change of the Gibbs free energy of the system, ΔG.

If the thermodynamic system is at constant temperature and pressure states, then the Gibbs free energy for spontaneous state change generally may be written as

$$-\Delta G = \Delta H - T\Delta S \tag{4}$$

with

$$\Delta H = \Delta(U + PV) \tag{5}$$

where ΔH is the energetic term consisting of internal energy U and the mechanical boundary work PV term. On the other hand, the term $T\Delta S$ expresses the probabilistic (statistical) aspects of the system, including the external magnetic field effect on the system, and may be expressed (through internal energy U change) as

$$T\Delta S = \Delta U + P\Delta V - \vec{H}\Delta\vec{M} \tag{6}$$

in which $\vec{H}$ is the strength of the applied magnetic field, $\vec{M}$ is the magnetization of the system and $\vec{H}$ is connected to $\vec{M}$ by

$$\chi = \frac{\vec{M}}{\vec{H}} \tag{7}$$

where χ is the magnetic susceptibility of the system.

Indeed in this case the change in Gibbs free energy is a direct measure of the tendency of a system under the influence of an applied magnetic field to perform the double helix-coil transition; that is, as temperature T of the DNA double helix in solution increases (applied magnetic field H is kept constant at already fixed P), bound (or double helix) pairs may unbind, thus yielding a coiled or single stranded state. The full significance of eqn. (4) in conjunction with eqns. (5—7) should be appreciated. For changes at constant P, T, $\vec{H}$ the spontaneity, or sign on ΔG will depend on the absolute values of ΔH and ΔS, that is, on the energetic and probabilistic factors of the system. Indeed, other things being equal, energy will tend to minimum; other things

being equal, entropy will tend to maximum.

The following ΔH and ΔS comparative balances in eqn. (4) may help to identify possible force interaction mechanisms responsible for retardation of the collapse of the secondary DNA double helix structure in solution under the influence of the applied magnetic field. If $\Delta H < 0$, $\Delta S > 0$, then $\Delta G < 0$. If $\Delta H > 0$, $\Delta S < 0$, then $\Delta G > 0$. However, if ΔH and ΔS have the same sign then the sign of ΔG will depend on the relative magnitudes of ΔH and $T\Delta S$ terms.

Assuming that both the native and the denatured DNA in solution have equal free energies at the double helix-coil transition temperature T_m (still dependent on environmental factors such as salt concentration, hydrogen ion activity, and base composition), eqn. (4) when applied to the transition state yields

$$\Delta H_m = T_m \Delta S_m \tag{8}$$

where ΔH_m is the transition heat of DNA in solution at transition temperature T_m; and ΔS_m is the corresponding double helix-coil transition entropy. Expressing eqn. (6) as the corresponding Gibbs free energy potential function

$$\Delta G = -S\Delta T + V\Delta P - \vec{M}\,\Delta\vec{H} \tag{9}$$

and combining eqns. (7), (8) and (9) for constant pressure, $\Delta P = 0$ process, we find that the corresponding temperature shift ΔT of the double helix-coil transition temperature T_m under the influence of the applied magnetic field H may be expressed in terms of the associated Gibbs free energy change ΔG per unit mass as

$$\Delta G = -\frac{\Delta H_m}{T_m}\,\Delta T + 1/2\,\Delta\chi(H\cos\psi)^2 \tag{10}$$

where, additionally, $\Delta\chi$ is the change in the magnetic susceptibility between the denatured and native states of the DNA molecule in solution at the transition temperature, T_m, and ψ is the angle between the applied magnetic field $\vec{H}$ and the direction of molecular orientation. Again, noting that at the double helix-coil transition $\Delta G = 0$, we can describe the melting point shift ΔT under the influence of an applied magnetic field as

$$\Delta T = \frac{T_m}{2\Delta H_m}\,\Delta\chi(H\cos\psi)^2 \tag{11}$$

The magnitude of the melting point shift ΔT is determined by the properties of the DNA double helix-coil transition in solution: T_m, ΔH_m and $\Delta\chi$, and the strength of the applied magnetic field $\vec{H}$ as well as the possible orientation of the DNA molecule with respect to the applied field.

We already know that the orientational angle $\psi = 0$ in solution at the double helix-coil transition temperature [47]. If we assume that the heat of the double helix-coil transition, ΔH_m, of calf thymus DNA in 0.0172 N KCl solution is 6.9 kcal $mole^{-1}$ of base pair [76], the average molecular weight of base pair is 650 [76]; but magnetic susceptibility χ may range from $\chi = -0.46 \times 10^{-6}$ emu g^{-1} [77,78] for DNA in aqueous solution, or $\chi = -0.54 \times$

10^{-6} emu g^{-1} for KCl aqueous solution [79,80] to that of $\chi = 100 \times 10^{-6}$ emu g^{-1} and larger χ values to account for possible magnetic state changes and ferromagnetic inclusion effects (see the preceding discussion) during the DNA double helix-coil transition in solution *, then substituting those listed values in eqn. (11), we may estimate the expected ΔT shift.

If we let $\chi_{native} = -0.46 \times 10^{-6}$ emu g^{-1}, but $\chi_{denaturation\ process} = 100$ emu g^{-1}, then through the use of eqn. (11), keeping the average molecular weight of base pair constant at 650, we obtain $\Delta T = 3.85 \times 10^{-3}$ K, indicating that transition temperature T_m under the influence of the applied magnetic field has increased. Increasing further the value of $\Delta\chi_{denaturation\ process}$ to $\approx 300 \times 10^{-6}$ emu g^{-1}, we obtain (for the same other conditions) $\Delta T > 0.1$ K. In general, the magnetic susceptibility range of 100×10^{-6} emu $g^{-1} \leqslant \Delta\chi \leqslant 300 \times 10^{-6}$ emu g^{-1} is characteristic of systems in which the magnetic interactions are caused by spin and usually orbital momentum of unpaired electrons. Such systems have also been observed during the DNA double helix-coil transition [56,81—87].

It should be recalled however that the presented relations are of qualitative nature. Certain caution should be displayed while interpreting these connections. Among these factors we note firstly that the observed $\Delta T = 0.1°C$ value is subject to an as yet unspecified experimental error; secondly, there are not available experimental values of magnetic susceptibility changes during the DNA double helix-coil transition in solution; thirdly, since eqn. (11) has been derived with respect to equilibrium state, the obtained ΔT-shift proportionality to the $\vec{H}^2$ term is strictly valid only at the close vicinity of T_m and it may well be of different behavior at high values of the applied magnetic field $\vec{H}$.

In terms of the well-known statistical DNA model of Poland and Scheraga [55] **, the total Gibbs free energy change [eqns. (4) and (9)] describing the DNA double stranded helix-single coil transition can be split into the following statistically weighted separate free energy terms [55] (see preceding discussions)

$$q = \exp(-G_{backbone}/RT) \tag{12}$$

$$k = \exp(-G_{side\ chain}/RT) \tag{13}$$

$$p = \exp(-G_{stacking}/RT) \tag{14}$$

$$t = \exp(-G_{interbase\ H\text{-}bonding}/RT) \tag{15}$$

$$\tau = \exp(-G_{double\ base\ stacking}/RT) \tag{16}$$

These separate Gibbs free energy terms [eqns. (12—16)] represent the following interactions [55]: $G_{backbone}$ is mostly entropy dominated term due to the internal rotation about the five covalent bonds in the backbone

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* At present there are no available experimental data regarding the changes of magnetic susceptibility during the DNA double helix-coil transition in solution.

** In addition to the already noted description of the DNA double helix-coil transition in solution by means of discrete steps [52—54], of interest to us is also Azbel's approach [88] in which he considers the DNA melting as a local phenomenon.

chain; $G_{side\ chain}$ dominated largely by the entropy of internal rotation about the sugar-base covalent bond; $G_{stacking}$ represents interactions between bases and the various solutes—solvents; $G_{interbase\ H\text{-}bonding}$ is controlled by an energy term; while finally $G_{double\ base\ stacking}$ represents mostly the overlap between neighboring base pairs.

Equation (6) indicates that the applied magnetic field effect will be noted on the DNA double helix-single coil transition in solution chiefly through the decrease of entropy. Indeed the above separate modes of interaction * [eqns.(12—16)] will contribute to the observed overall retardation of the DNA double helix—single coil transition under the influence of the applied magnetic field H (Figs. 3—5) in terms of the relative decrease of their entropy values. The importance of the entropy decrease in retarding the DNA denaturation process in solution has been brought out indirectly by calculating the ΔT shift [eqn. (11)] as well as the indicated (see the previous discussion) distortion of backbone of the DNA molecule by the applied magnetic field H due to the induced changes in bond stretching and bending.

Considering the complex nature of the many simultaneously occurring interactions during the thermal denaturation process of the DNA double helix in solution, the exact reason for the observed efflux time increase (Figs. 3—5) of calf thymus DNA in solution under the influence of the applied magnetic field H, at least at this time cannot be decided with any certainty. A more detailed explanation of the observed magnetic-induced change in the efflux time must await further elucidation.

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