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Symmetry of electrostatic interaction between pyrophosphate DNA molecules

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Abstract. We study chiral electrostatic interaction between artificial ideal homopolymer DNA-like molecules in which a number of phosphate groups of the sugar-phosphate backbone are exchanged for the pyrophosphate ones. We employ a model in which the DNA is considered as a one-dimensional lattice of dipoles and charges corresponding to base pairs and (pyro)phosphate groups, respectively. The interaction between molecules of the DNA is described by a pair potential U of electrostatic forces between the two sets of dipoles and charges belonging to respective lattices describing the molecules. Minima of the potential U indicate orientational ordering of the molecules and thus liquid crystalline phases of the DNA. We use numerical methods for finding the set of minima in conjunction with symmetries verified by the potential U. The symmetries form a non-commutative group of 8th order, $\mathcal{S}$. Using the group $\mathcal{S}$ we suggest a classification of liquid crystalline phases of the DNA, which allows several cholesteric phases, that is polymorphism. Pyrophosphate forms of the DNA could clarify the role played by charges in their liquid crystalline phases, and open experimental research, important for nano-technological and bio-medical applications.

1 Introduction

According to the familiar legend, the discovery of the cholesteric phase of DNA was due to a happy chance that occurred to C. Robinson, who was sharp enough to see it [1]. The story resembles that of Fleming discovering the penicillin. Since then, cholesteric phases of DNA have been the subject of numerous experimental and theoretical investigations owing to their variety and regularity [2]. It has been established that the formation of the phases depends on the chemical properties of an ambient solution and ions, the ingenious experimental technique has been worked out in [2,3], and liquid crystalline phases of the DNA have become instrumental in studying the DNA itself. Another important development began surfacing in chemical physics of nucleic acids early in the '90s. The group led by Z.A. Shabarova at the Lomonosov Moscow University [4–8] succeeded in synthesizing the DNA in which some internucleotide phosphate groups are exchanged for the pyrophosphate ones, and thus considerably extended the field of research, providing new insights into the chemistry of nucleic acids, as well as new possible bio-medical applications. In this paper we aim at making it clear that pyrophosphate forms of the DNA could be helpful in studying liquid crystalline phases of the DNA.

Theoretical work on the physics of liquid crystalline phases dates from the seminal paper by Onsager [9], which relies on the picture of hard rods representing molecules in solvent. Applications of the model require the use of phenomenological constants and theoretical assumptions, difficult to verify in specific situations. Cholesteric phases need even more careful investigating, owing to their chirality. In a series of papers Kornyshev, Leikin, and their collaborators [10–13], put forward the theory of cholesteric liquid crystalline phases of the DNA that relies on the helical distribution of charges of the DNA. Within the framework of this theory, one can employ various approaches and approximations and investigate specific conformations. Generally, a molecule of the DNA is considered as a charged rod or cylinder, the charge being distributed continuously over the surface of the rod, complying with the helical symmetry, theoretical tools employed being of analytical character. In the present paper we use a discrete approximation for the charge distribution and employ a computer simulation for finding molecular conformations. It should be noted that the distribution of charge in the DNA molecule is essentially discrete being caused

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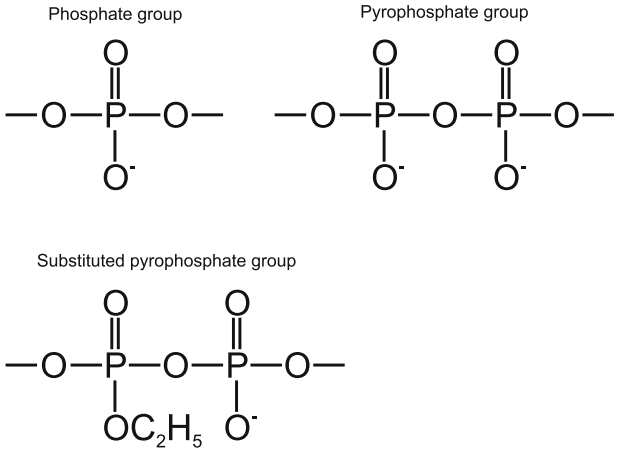

Fig. 1. Phosphate, pyrophosphate and substituted pyrophosphate groups.

by 1) dipole moments of the base pairs; 2) charges of the phosphate groups, 3) counterions which are not uniformly distributed around the DNA molecule. The electrostatic interaction between two DNA molecules is due to this essentially non-uniform distribution of charges. Our approach, still remaining within the framework of papers [10, 11,14–16] (see also the very recent paper [17], containing an extended overview of experimental and theoretical achievements regarding chiral electrostatic DNA-DNA interactions) provides new details of the phenomenon. It is worth noting that we aim only at a qualitative description, which could be useful for explaining experimental data.

Since the current theory considers electrostatic interaction as a cause for the formation of liquid crystalline phases of the DNA, it is interesting to investigate opportunities that can be provided by the use of DNA containing a number of pyrophosphate groups, PP forms, instead of the usual phosphate ones, P forms, see fig. 1, so as to have a means of changing the charge conformation of the molecule. It is important that synthetic forms of the DNA can contain PP groups in the duplex of the DNA instead of the usual phosphate ones in such a way that the structures of the pyro-modified and usual phosphate molecules remain rather close [8], the internucleotide distance, the stacking, and the Watson-Crick interaction suffering no change.

Synthetic forms of the DNA are instrumental in the study of fundamental problems in molecular biology, biochemistry, medicine, ferments' activity in nucleotide exchange, protein-nucleic acids complexes, structural functioning of biopolymers, and regulation of the genetic expression. The modification of internucleotide groups is of particular importance owing to its preserving the ability of molecules of the DNA to penetrate cell membranes and regulate gene expression, while retaining the basic function of the DNA, that is to interact with the complimentary sequences of nucleotides. It is possible to synthesize the DNA so as to have the exchanged pyrophosphate groups located at prescribed sites of the sugar-phosphate spine, each pyrophosphate group bringing forth an additional negative charge.

Minima of the potential U for pair interaction of molecules of DNA should correspond to orientational ordering of the molecules in solvent and therefore liquid, or solid, crystalline phases. Special means are required to find the minima of U. At this point the symmetry of U provides valuable information. As was found in the previous paper [18], U is invariant under the action of a group of discrete transformations, and therefore its minima form a set having the same symmetry. The circumstance reduces the amount of numerical work, which is quite large. But we feel that the symmetry of the pair interaction U is by far of more general importance for understanding the physics of liquid crystalline phases of the DNA than one can infer from its numerical applications.

2 Preliminaries

We shall recall certain basic facts of DNA. A molecule of DNA can attain several hundred μm in length. If we neglect details that have a size of one thousand Å, or more, we can visualize it as a soft shapeless line and conclude that on this scale it behaves like an ordinary polymer. In contrast, looking at its smaller segments, of one hundred Å or less, we observe that it tends to be straight. Thus, borrowing a comparison from everyday life, we may say that a molecule of the DNA looks like a piece of steel wire whose long segments are flexible and the short ones are stiff. The elastic properties of the DNA are intimately related to its being a double helix. The latter imposes severe constraints on deformations which can be effected without destroying the molecule and to a large extent determines its mechanical properties. In fact, the two strands comprising the molecule of DNA have just small bending rigidities, just as usual polymers. But the formation of the two-stranded structure drastically changes the DNA by making it both stiff and capable of forming sophisticated spatial shapes.

As was mentioned above, the double helix of DNA consists of long chains, or strands, which have the backbones composed of sugar and phosphate residues, and special chemicals, bases, keeping the two strands together (the structure is illustrated in fig. 2). The fundamental building blocks of the strands are nucleotides, joined to each other in polynucleotide chains. The nucleotide consists of a phosphate joined to a sugar (2′-deoxyribose), to which a base is attached. The sugar and base alone are called a nucleoside. The chains, or strands, of the DNA wind round each other in a spiral forming a double helix, the bases being arranged in pairs: adenine-thymine (AT), guanine-cytosine (GC), so that the sequence of bases in one strand determines the complimentary sequence of bases in the other and constitutes the genetic code stored by the molecule of DNA. There are several forms of DNA, denoted by A, B, and Z. The most common one in nature is the so-called B-form. One turn of the helix of the B-form corresponds approximately to 10.5 base pairs, and the distance between adjacent pairs of bases is approximately 3.4 Å. In real life there are considerable deviations from the canonical B-form of the DNA. Therefore, there is a need for a special nomenclature for describing its conformations

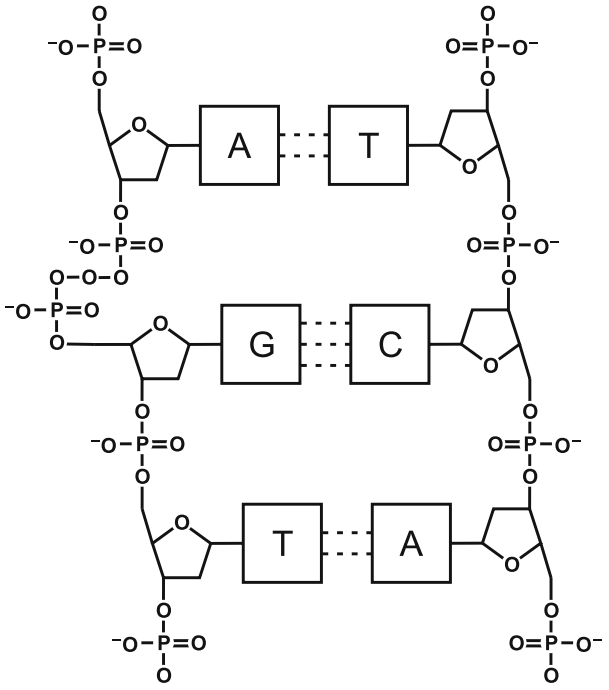

Fig. 2. DNA with a pyrophosphate group.

(see [19] for the details), and generally a considerable set of parameters is required. It is worth noting that the deviations from the canonical form are by no means small, and may have a size of tens of degrees.

Synthetic analogs of nucleic acids (NA) containing modified internucleotide groups are useful for solving various problems of molecular biology, biotechnology, and medicine. Shabarova *et al.* [4,5], developed a novel type of modified DNA duplexes containing pyrophosphate (PP) and substituted pyrophosphate (SPP) internucleotide groups at the definite position of the sugar-phosphate backbone [4–8] (see fig. 1). The PP group bears one additional negative charge in comparison with a natural internucleotide group; the SPP group contains no additional charges. The introduction of PP groups into DNA leads to an increase of the total negative charge of a molecule of the DNA. The study of oligonucleotide duplex containing a PP and SPP groups has revealed that stacking and Watson-Crick interactions are not significantly affected. By flipping out of the disubstituted phosphate, these groups fit into the helix structure without elongation of the internucleotide distance. The analysis of helical parameters of base pairs, internucleotide distances, and overall global structure, reveals a close similarity of the initial and modified duplexes.

The location of PP groups of the DNA have to verify certain conditions:

1) their total number does not exceed 10% of the total number of phosphates;
2) they are not allowed to be located opposite each other;
3) they are not allowed to occupy the ends of a molecule;
4) two adjacent pyrophosphate groups are to be separated by at least 10 phosphate ones.

At the time of writing this paper there are no experimental investigations of pyrophosphate DNA self-organization. The richness of the various DNA forms organizations including different liquid crystalline phases (see, *e.g.*, [20,21]) depend on a number of physical properties, such as DNA concentration, DNA length, DNA molecular form and charge distribution, type of counterions, their concentrations, and also concentration of incompatible polymer. In our simple model all these relevant quantities are lumped into a few effective parameters, like screening radius, charge and dipole distributions. One might expect that uncorrelated charge distribution patterns, due to pyrophosphate substitutions, distort DNA-DNA register and therefore suppress the tendency to liquid crystalline ordering. Indeed, relatively strong azimuthal correlations are necessary for the existence of pyrophosphate DNA cholesteric liquid crystals. Using as a criterion of the pyrophosphate suppression of cholesteric order, the condition that the energy to rotate azimuthally a pyrophosphate DNA as a whole around its axis is of about the thermal energy k_BT, we anticipate, based on our calculations, that the structures predicted in our paper can be observable and that understanding of the underlying mechanisms will be essential to predict the behavior.

3 Model

Theoretical study of liquid crystalline phases of the DNA generally uses models that are necessarily based on very crude simplifications. The first point at issue is the right choice of the potential of the interaction. In this paper we model the DNA molecules as a 1-dimensional lattice of charges and dipoles with an elementary cell of size 3.4 Å. It mimics the spatial conformation of charges and dipoles of phosphate groups and base pairs. We consider short segments of the DNA, approximately 500 Å, that is of the size of persistence length, so that to a good approximation they are segments of straight lines, and assume that both molecules have the same number, 151, of base pairs that can be visualized as points on a straight line parallel to the axis of the molecule, one base pair being located at the center of a corresponding molecule (see fig. 3). The centers of the straight lines belong to a straight line perpendicular to x-y plane which is parallel to either of them. We shall denote by ξ the angle between the straight lines describing the molecules. Both molecules are of the same helicity, which is determined by the rotation of the frame of the dipole moments. Thus, we model a molecule of DNA on a one-dimensional lattice having at its sites either vectors of dipoles and scalar charges of the base pairs and the phosphate. It is important that the values of the dipoles and charges are renormalized owing to screening effects caused by counterions and ions adsorbed at the molecule. Therefore, we consider effective charges and dipoles moments. The case of total neutralization of phosphate charges was considered in paper [16].

One of the main difficulties in comparing the results of our effective and simplified model with specific experimental measurements (see, *e.g.*, on optic, X-ray and NMR

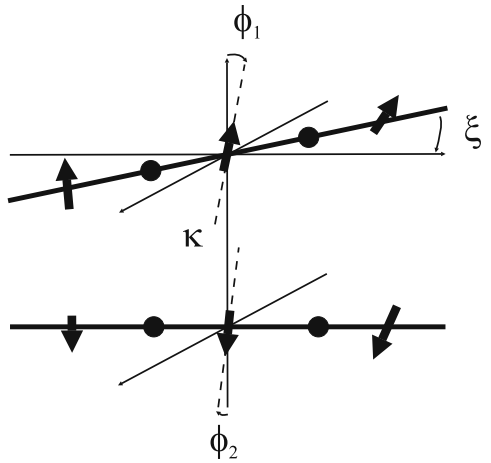

Fig. 3. Scheme for the locations of dipoles and charges of base pairs and P groups, respectively, in a molecule of DNA. The conformation of a pair of molecules is determined by the angle ξ between the axes of the molecules, and angles ϕ_1, ϕ_2 of rotations of the molecules about their axes.

investigations [22–26]) or elaborated microscopic numerical simulations (see, *e.g.*, [27–30]) is the availability of an accurate connection between experimental control parameters and theoretical model coefficients. The actual values of the parameters are determined by the microscopic (atomic level) interactions. Since these interactions are not well known, we regard the values of the effective charges and dipole moments as phenomenological coefficients. In this sense our model should be treated as a working hypothesis. Comparison of the qualitative predictions resulting from this hypothesis with experimental or microscopical numerical observations will show whether and when this hypothesis is justified. Following this logic, without prior knowledge of the actual values of multipole moments for the DNA charge distribution, we assume the simplest model, where all electrostatic interactions physics is lumped into the effective charges and dipoles. At the time of writing the nature and significance of higher-order multipoles and tricky details of charge screening and condensation are still far from being clear. Note also that the interaction energy due to higher-order multipole moments decays rather fast with intermolecular distance and should not qualitatively affect our results mainly relevant for relatively dilute systems. Since the most general form of the DNA molecule interactions is exceptionally tedious and intractable by simple numerical means and also for convenience and because of the lack of different compelling indications, we restrict ourselves to this minimal model. Although it provides some insight into the mechanisms of DNA aggregation phenomena, we are left with many questions unanswered which must be pursued in further work.

The dipoles are suggested to have the helix symmetry with $\pi/5$ rotation/bp, corresponding to the structure of the ideal double helix of the DNA. Of course, it is necessary to take into account the structure of DNA being not uniform and the relative positions of the base pairs varying slightly from base pair to base pair. Hence, the dipole moments do not form a precise lattice structure. Even more so they should depend on the local DNA sequence. Therefore, our assumption of the regular dipole positions is a crude *approximation*.

The distance, κ, between the centers of the lattices, which is fixed, is an important parameter of the model. In what follows we use the distance between adjacent base pairs, that is 3.4 Å, as a unit of length, take a unit of charge for which the dipole moment of 1 Debye equals 1, and perform calculations in the dimensionless units generated by these quantities.

The energy of the electrostatic interaction of two molecules can be cast into the sum

$$\epsilon U = U_0 + u_{dd} + u_{dc} + u_{cd} + u_{cc}, \tag{1}$$

in which ϵ is the dielectric permeability of solvent and U_0 is the self-energy of the pair, which does not influence its conformation; the first term describes the interaction between dipoles of the first molecule and those of the second; the second term the dipoles of the first and phosphate charges of the second; the third the charges of the first and dipoles of the second; the fourth the charges of the first and the second. The interactions are given by the equations

$$u_{dd}(\rho) = e^{-\nu\rho}\left[g(\rho)\frac{1}{\rho^3}(\mathbf{p}\cdot\mathbf{p}') - 3h(\rho)\frac{[\mathbf{p}\cdot(\mathbf{r}-\mathbf{r}')][\mathbf{p}'\cdot(\mathbf{r}-\mathbf{r}')]}{\rho^5}\right], \tag{2}$$

$$u_{dc}(\mathbf{r},\mathbf{r}') = e^{-\nu\rho}k(\rho)Q'\frac{\mathbf{p}\cdot\mathbf{r}'}{\rho}, \tag{3}$$

$$u_{cd}(\mathbf{r}',\mathbf{r}) = e^{-\nu\rho}k(\rho)Q\frac{\mathbf{p}'\cdot\mathbf{r}}{\rho}, \tag{4}$$

$$u_{cc}(\mathbf{r},\mathbf{r}') = e^{-\nu\rho}\frac{QQ'}{\rho}, \tag{5}$$

in which ν is the inverse Debye length $\nu = \lambda^{-1}$, and

$$\rho = |\mathbf{r}-\mathbf{r}'|.$$

We shall take the screening functions $k(\rho)$, $g(\rho)$, $h(\rho)$ in Schwinger form

$$k = g = 1 + \nu\rho, \qquad h = 1 + \nu\rho + \frac{1}{3}\nu^2\rho^2.$$

The important point about the electrostatical interaction between molecules of the DNA is a wise choice of the screening factor. The common practice is to employ the Debye-Hückel theory, or its modifications that might accommodate the dipole charges, the so-called Schwinger screening [31]. The full treatment of this problem requires a separate investigation. In this paper we confine ourselves to the Debye-Hückel and the Schwinger theories [31].

It is worth noting that the pair potential U is invariant: if we change the sign of the angle ξ between the axes of the two molecules, at the same time as the sign of helicity,

the potential U remains the same. There are symmetry rules for the helices of the same kind. One may convince oneself that the following transformations

$$t_1 : (\phi_1, \phi_2, \xi) \to (\phi_1, \pi - \phi_2, \xi + \pi), \tag{6}$$
$$t_2 : (\phi_1, \phi_2, \xi) \to (\pi - \phi_1, \phi_2, \xi + \pi), \tag{7}$$
$$t_3 : (\phi_1, \phi_2, \xi) \to (\phi_2 + \pi, \phi_1 + \pi, \xi), \tag{8}$$

leave the potential U invariant. The angles are defined within limits

$$-\pi \le \phi_1 \le \pi, \qquad -\pi \le \phi_2 \le \pi, \qquad -\pi \le \xi \le \pi,$$

values $\pm\pi$ corresponding to the same configurations of the molecules. The transformations given by eqs. (6)-(8) verify the equations

$$t_1^2 = t_2^2 = t_3^2 = id, \qquad t_2\, t_3 = t_3\, t_1, \qquad t_1\, t_2 = t_2\, t_1,$$

where id is a transformation that leaves all ϕ_1, ϕ_2, ξ invariant. Using the above equations one can easily convince oneself that t_1, t_2, t_3 generate a *non-commutative group* of 8th order, $\mathcal{S}$. Its maximal subgroup $\mathcal{H}$ is a normal subgroup of 4th order, commutative, and generated by the transformations

$$f_1 = t_3, \qquad f_2 = t_1\, t_2\, t_3. \tag{9}$$

Elements f_1, f_2 in their turn generate subgroups $\mathcal{H}_1$ and $\mathcal{H}_2$ of $\mathcal{H}$, respectively. It is worth noting that $\mathcal{H}_1$, $\mathcal{H}_2$ are of second order, both. They are conjugate subgroups of $\mathcal{S}$, that is for an element g of $\mathcal{S}$ we have $f_1 = g^{-1} f_2\, g$, or we may state $\mathcal{H}_1 = g^{-1}\, \mathcal{H}_2\, g$, in the notations of group theory, which can be cast in the form of the diagram

$$\mathcal{H}_1 \longleftrightarrow \mathcal{H}_2. \tag{10}$$

The element

$$f_3 = t_1\, t_2 \tag{11}$$

generates subgroup $\mathcal{H}_3$ of $\mathcal{H}$. It is important that $\mathcal{H}_3$ is a normal subgroup of $\mathcal{S}$, that is $g^{-1}\, \mathcal{H}_3\, g = \mathcal{H}_3$ for any element g of $\mathcal{S}$. Thus, we have the diagram of subgroups inside the symmetry group $\mathcal{S}$:

$$\begin{array}{ccccc} \mathcal{H}_1 & & & & \\ & \searrow & & & \\ \mathcal{H}_3 & \longrightarrow & \mathcal{H} & \longrightarrow & \mathcal{S}, \\ & \nearrow & & & \\ \mathcal{H}_2 & & & & \end{array} \tag{12}$$

in which the arrows signify the embedding of subgroups.

The group of symmetries, $\mathcal{S}$, plays the key role in finding the minima of the potential U. The following general arguments, based on the theory of groups, are quite useful in this respect. Consider a point μ of space $\mathcal{X}$ of the angles ϕ_1, ϕ_2, ξ. Suppose that μ is a minimum of U. Then, points

$$\mu' = g \cdot \mu,$$

called the orbit of the point μ under the action of the group $\mathcal{S}$, are also minima of U. The number of points μ' of the orbit can vary. In fact, let us consider all transformations g of $\mathcal{S}$ that leave μ invariant, that is $\mu' = g \cdot \mu = \mu$. It is alleged to be known that the transformations form a subgroup of $\mathcal{S}$, called stationary subgroup $\mathcal{H}_\mu$. The stationary subgroups, $\mathcal{H}_\mu$ and $\mathcal{H}_\nu$, for points μ and ν of an orbit, are conjugate, that is $\mathcal{H}_\mu = g^{-1}\, \mathcal{H}_\nu\, g$ for an element g of $\mathcal{S}$. The number of different points μ' equals to the ratio of the orders of $\mathcal{S}$ and $\mathcal{H}_\mu$, that is to 2 or 4, depending on the choice of the point μ. To be specific, consider a point μ having a stationary subgroup $\mathcal{H}_\mu$ that coincides with the subgroup $\mathcal{H}$. The latter is a normal subgroup of $\mathcal{S}$ of index 2, that is the factor set $\mathcal{S}/\mathcal{H}$ consists of two elements. Thus, the orbit of μ under the action of $\mathcal{S}$ consists of only two points that correspond to the same value of U and have the same stationary subgroup $\mathcal{H}$, because the latter is a normal subgroup of $\mathcal{S}$. The situation is quite different if we take a point ν having stationary subgroup $\mathcal{H}_1$, which is different from $\mathcal{H}_2$. The subgroups do not coincide in $\mathcal{S}$, even though they are conjugate. The orbit of μ under the action of $\mathcal{S}$ indicated above consists of four points that we may sort out as follows: two points having the stationary subgroup $\mathcal{H}_1$ and two points having $\mathcal{H}_2$. This is due to the fact that for one thing the subgroup $\mathcal{H}$ is commutative and therefore its elements generate points of the orbit but with the same stationary subgroup, that is $\mathcal{H}_1$, and for another there is an element g that gives points of the orbit having the stationary subgroup $\mathcal{H}_2$. In contrast, a point μ having the stationary subgroup $\mathcal{H}_3$ has the orbit consisting of four points which have the same stationary subgroup $\mathcal{H}_3$, because the latter is a normal subgroup of $\mathcal{S}$.

4 Numerical simulation

It is to be noted that the numerical evaluation of the minima runs across a poor convergence of standard algorithms for minimization, owing to flat surfaces of constant value for the function of three variables, $U(\phi_1, \phi_2, \xi)$. To some extent, one may get round the difficulty by observing that for points remaining fixed with respect to a subgroup $\mathcal{G}$ of $\mathcal{S}$, the minimization problem is reduced to that for a smaller number of variables. This is due to the necessary conditions for the extremum being verified automatically for degrees of freedom perpendicular to the set of invariant points, so that one needs only to study the conditions for longitudinal variables, that is to solve a smaller system of equations. For numerical calculations we are utilizing the gradient descent algorithm usually applied to find minima for poorly converging functions, like for instance the electrostatic energy (1) depending on three angular variables. Unfortunately even this algorithm provides a rather low accuracy of about 0.01 for regions close to degeneracy where the second derivative of (1) takes very small values. However, aiming only at a qualitative clarification of the symmetry of electrostatic interactions, we believe that, although our results concerning the minima positions may not have exact numbers, they have more or less the right shape.

To see the point let us consider a function $f(x, y, z)$ of variables x, y, z even in x, so that $f(x, y, z) = f(-x, y, z)$.

The set of invariant points is the y-z plane, and we may look for minima of the function $f(x = 0, y, z)$, thus we need to solve only two equations

$$\frac{\partial}{\partial y} f(x = 0, y, z) = 0, \qquad \frac{\partial}{\partial z} f(x = 0, y, z) = 0.$$

The number of variables necessary for calculations can be reduced even further in case of larger groups of symmetries. It is easy to convince oneself that the sets of fixed points (ϕ_1, ϕ_2, ξ), that is invariant under the action of a subgroup of $\mathcal{S}$, read as follows:

$$\mathcal{F}_1 : (\phi_1 = \phi, \phi_2 = \phi + \pi, \xi), \tag{13}$$

$$\mathcal{F}_2 : (\phi_1 = \phi, \phi_2 = -\phi, \xi), \tag{14}$$

$$\mathcal{F}_3 : \left(\phi_1 = \pm\frac{\pi}{2}, \phi_2 = \pm\frac{\pi}{2}, \xi\right), \tag{15}$$

in which the $\mathcal{F}_i$'s are invariant under the action of subgroups $\mathcal{H}_1$, $\mathcal{H}_2$, $\mathcal{H}_3$, respectively. The above symmetries are illustrated in fig. 4.

The analysis of symmetries of U given above, see sect. 3, enables us to sort out the minima according to the effective value of the phosphate charge Q. The dependence of the values of minima on the effective charge is illustrated in fig. 5. Features of the synthesis of PP-modified DNA molecules are such that the PP groups cannot be situated too close to each other. The minimum distance between two PP groups in the synthesized molecules is of the order of ten base pairs, which implies that the total content of these groups cannot exceed a few percent. In addition, the PP groups must be spaced at no less than 5 base pairs from the ends of a molecule [5,8]. With all this in mind, in our studies we have used the following typical conformations:

i) PP groups are arranged symmetrically at the ends of molecules;
ii) PP groups are arranged symmetrically at the centers of molecules;
iii) PP groups are uniformly distributed along the molecules.

The results of computations for some combinations of these conformations are shown in fig. 5 (as concerns the minima of the interaction energy) and in fig. 6 we plotted the cholesteric twist angle *versus* the effective charge. The results of our simulations are indicative of polymorphism, that is, the existence of various cholesteric and nematic phases in solutions. This conclusion follows from the presence of various minima in the pair interaction potential. In this sense we have to consider structures with zero twist angle as nematic liquid crystals. This transition (to zero twist angle or to infinite pitch with effective charge as a controlling parameter) phenomenologically could be a continuous (second-order) phase transition, although its consideration is beyond our primitive mechanical model.

It should be noted that the minima of the pair interaction U depend on the distance between molecules κ, and the effective phosphate charge Q. The latter is the control parameter we employ in numerical simulation. It is also

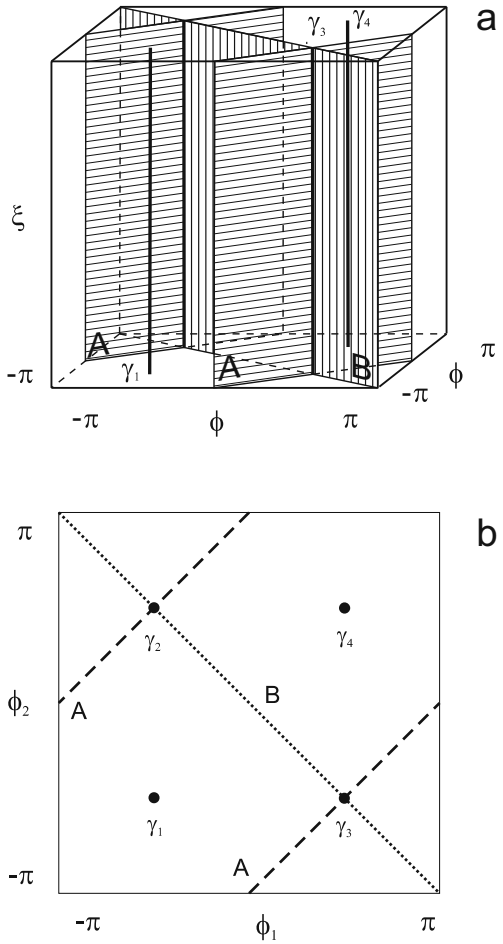

Fig. 4. (a) Cube of the symmetries indicating the sets in space ϕ_1, ϕ_2, ξ invariant with respect to the subgroups of $\mathcal{S}$. Main diagonal plane, B, corresponding to subgroup $\mathcal{H}_2$; two rectangles, A, perpendicular to B, correspond to $\mathcal{H}_1$; solid lines γ_1, γ_4, and γ_2, γ_3 corresponding to $\mathcal{H}_3$ and $\mathcal{H}$, respectively. (b) Cube of the symmetries, view from the top. Dotted line describes the invariant points $\mathcal{F}_2$, corresponding to subgroup $\mathcal{H}_2$; dashed line points $\mathcal{F}_1$ and subgroup $\mathcal{H}_1$; solid circles γ_1 and γ_4 to subgroup $\mathcal{H}_3$; γ_2 and γ_3 to subgroup $\mathcal{H}$.

useful for the description of possible experimental results. In this paper we are considering κ to within 10.2 ± 3.4 Å. The effective charge Q of the phosphate groups determines the neutralization; it varies to within 0 ± 0.6, in dimensionless units, $Q = 0$ corresponding to the total neutralization. The charges Q that correspond to the charge inversion have not been considered. The Debye length, λ, has been varied to within 7 ± 3.5 Å, depending on the ion strength of the solution.

The value of the effective charge Q is determined by its electrostatic surrounding. It depends not only on ion charges in solvent, but also on those adsorbed by a molecule of the DNA. It seems that the conventional Debye-Hückel theory does not work in this situation [31]. At any rate, it does not accommodate the adsorbed charges. According to [3], the effective charge is small.

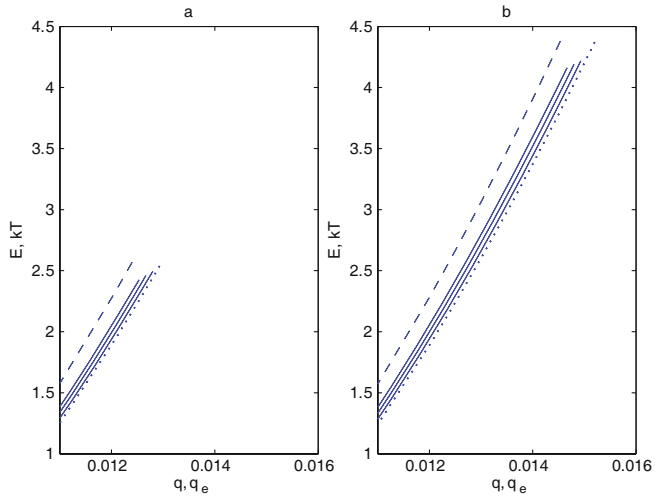

Fig. 5. Minima of U in units of k_BT, $T = 300$ K: (a) cholesteric angle $\xi > 0$; (b) $\xi < 0$; against effective charge q in units of the electron charge. Values of $U(\xi, \phi_1, \phi_2)$ and $U(-\xi, \phi_1, \phi_2)$ are equal to within $0.01k_BT$. Dotted lines correspond to the case of molecules free of PP groups, dashed lines represent the case of PP groups uniformly distributed along the molecule, solid lines refer to the molecules with symmetrically arranged PP groups. Minima that could correspond to the cholesteric phase with $\xi > 0$ vanish at $q = 0.013$.

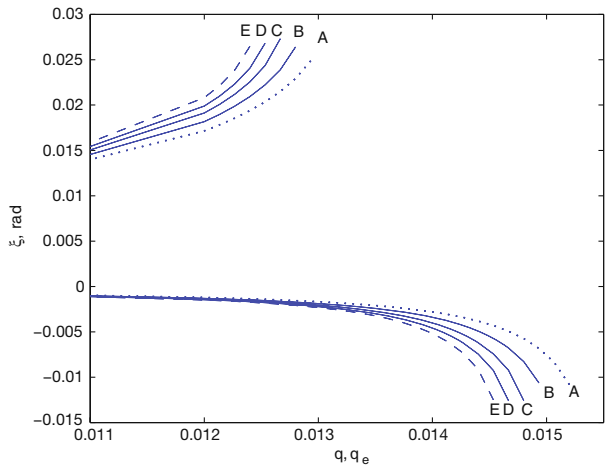

Fig. 6. Cholesteric angle ξ against effective charge q in units of the electron charge. PP groups are located symmetrically: (A) no PP groups; (B) one PP group at either end of a molecule; (C) two PP groups at either end of a molecule; (D) three PP groups; (E) every tenth P group is exchanged for the PP one.

The numerical data and the symmetry analysis given above suggest that there should be the following three types of minima:

1) Type I, characterized by molecules having a cross-like conformation, "snowflakes", that is ξ being close to $\pi/2$. It exists for Q large enough. Its symmetry subgroup depends on Q and may take values $\mathcal{H}_1$, $\mathcal{H}_2$, $\mathcal{H}_3$, $\mathcal{H}$. Therefore, we may claim that there exist four subtypes of minima I: $I_{\mathcal{H}_1}$, $I_{\mathcal{H}_2}$, $I_{\mathcal{H}_3}$, $I_{\mathcal{H}}$, each of them consisting of two subtypes which are given by specific conformations of the angle variables.
2) Type II, for which ξ takes values to within 0.1°; the symmetry subgroups are $\mathcal{H}_1$ and $\mathcal{H}_2$, either type consists of two subtypes.
3) Type III, for which ξ is to within 1°, that is larger than for type II. The symmetry subgroup is $\mathcal{H}_3$, and there are four constituent types of the same symmetry.

As can be inferred from the considerations given above, the study of the pair interactions between molecules of the DNA requires a means to vary the charges of a molecule and their positions in it. By now the only method available to that end is to vary the ion composition of the solvent, the molecules itself being intact. The use of the DNA containing PP groups should provide new opportunities for the research, for it could change in a prescribed way the conformation of charges of the molecule. Thus one may compare the formation of liquid crystalline phases for the same solutions but for a different charge conformation of the DNA. Our numerical simulation suggests that the effect could be tangible enough to be seen in experiment. The dependence of the minima on the effective charge taking into account PP groups is indicated in fig. 6. The behavior of U is illustrated in fig. 7 by means of iso-energy surfaces. Another point in favor of working with the pyrophosphate forms of the DNA is that one can vary the effective charges of a molecule. In the case of the usual phosphate DNA, the phosphate charges are all equal, and therefore one may suggest that the effective charges, which enter in our simulation, are also equal. Using the pyrophosphate forms, we may expect to achieve even the regime for which all the phosphate charges are neutralized whereas the pyrophosphate ones still remain, even though being small. Such an experiment would be helpful in determining the nature of intramolecular pair interaction that leads, according to the accepted physical picture [10], to the formation of cholesteric phases of DNA.

The main point about our numerical simulation is the choice of the values for effective charges and dipoles. In case there are PP groups, we may consider the effective charges in the way described above. The situation is more subtle as far as dipoles are concerned. We assume their numerical values to be of first order in the units indicated above. This is as much as to say that the charges of the base pairs that constitute dipoles are screened much less than the phosphate ones. If we proceed otherwise and take small values of the dipoles, there are no small minima but a non-zero value of ξ, that is no cholesteric phases. However, cholesteric structures have been observed in experiments [2]. Thus, it is reasonable to assume that the charges of base pairs are screened in a manner different from that for the phosphate ones. One may suggest that the renormalization of charges is due mainly to adsorption of ions from the solvent, and not to the screening clouds of ions in the solvent. If so, it is likely that the charges of P groups, due to ions of O^-, are in a different position than those of the base pairs, and the renormalization of charges of P groups and base pairs follow different paths. For this argument we are indebted to Yu. M. Yevdokimov.

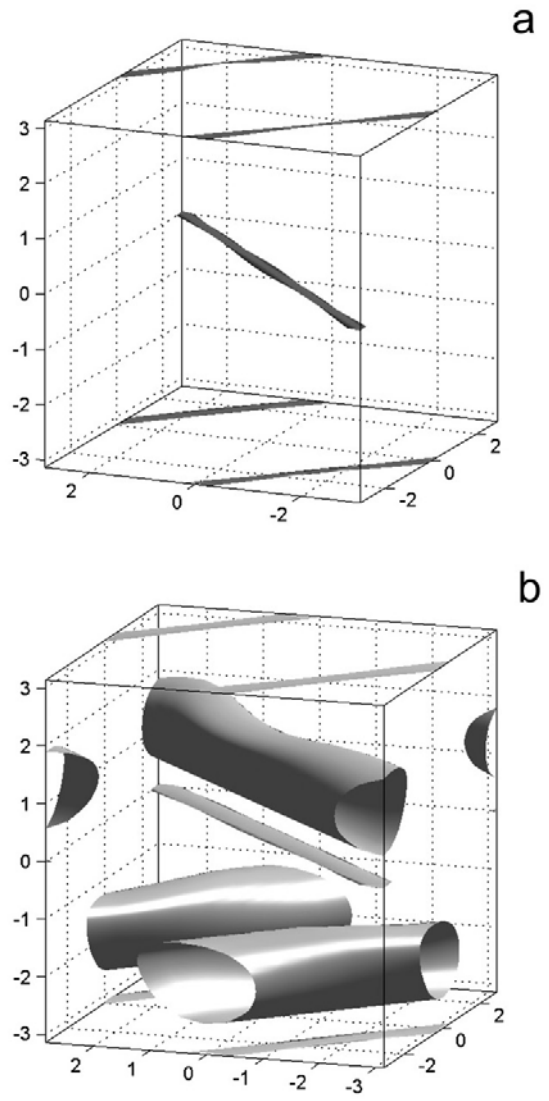

Fig. 7. Surfaces of constant value of U near cholesteric minima. (a) Screened charge $q = 0.0086q_e$, the energy level is $0.05kT$. (b) Screened charge $q = 0.0086q_e$, the energy level is $0.2kT$.

The important point is that PP groups influence the formation of cholesteric angles different from those of P groups, and thus provide a means of identification of new phases. Summing up:

- the phase of "snowflakes" sustains the presence of PP groups;
- the PP groups may result in splitting energy levels of minima, so that minima corresponding to the same values of U become different, when PP groups are present;
- the minima of U are separated by low potential barriers; iso-energy surfaces of constant values of U being like galleries between halls that illustrate the minima;
- the minima corresponding to cholesteric phases are very narrow, whereas those of snowflakes are broad and sloping; the energy barriers separating minima corresponding to snowflakes and cholesteric phases, respectively, are of the order k_BT;
- the values of the angles ϕ_1, ϕ_2 are subject to constraints inside galleries joining two minima.

5 Conclusions: New opportunities for studying the liquid crystalline phases of the DNA

Summarizing, in this paper we have investigated certain peculiar symmetry features of pairwise electrostatic interactions between two ideal DNA homopolymer rods. The coupling brings about electrostatic frustrations, which in turn results in a rich variety of chiral orientational energy minima. The relatively long-range nature of not too strongly screened charge and dipole electrostatic interactions presents a multitude of configurations that are good minimum-energy candidates also for condensed many-particle phases, and yet far apart in the configurational space. We have focused only on electrostatics. Hard-core forces are clearly important, and in many cases their contribution is dominant. Chiral torques might be also produced by the van der Waals and hydration forces. In our paper we neglect completely all these contributions. van der Waals forces are typically much smaller than the electrostatic ones, and hydration and steric forces are short ranged and relevant only for dense structures. Aiming to an only qualitative, mainly symmetry classification, picture, we do believe that although our results may not have exactly the right numbers, they have more or less the right symmetry and scaling shape. Furthermore, we analyze the electrostatic energy, rather than the free energy. Because we are interested in relatively short and rigid DNA segments, their internal conformational entropy could be safely neglected. The translational entropy of aggregates formed by many DNA molecules is beyond the scope of our paper devoted to the only pairwise interaction of two DNA segments at a fixed distance between their centers of mass.

The pyrophosphate DNA may serve a valuable probe into the physics of cholesteric phases of DNA, providing a unique opportunity for changing the effective charge of a molecules of DNA. The use of PP-forms may result in appreciable experimental effects, which in its turn could throw light on the nature of intramolecular interaction in the solution of DNA. The charge screening still poses a number of questions. The usual Debye-Hückel theory does not seem to be an adequate solution [31], especially as the screening of electrical dipole moments is concerned. A theory that could give a reasonable agreement with experiment should be that of finite number of particles, whereas the Debye-Hückel theory relies on the use of macroscopical considerations. The study of the cholesteric phases of DNA with pyrophosphate internucleotide insertions could provide a means to find characteristics that indicate a way to understanding the phenomenon. An important issue is the different screening of the phosphate charges and the dipoles of the base pairs. It is should be noted that the screening is caused by a non-uniform adsorption of counterions at the molecule of DNA, so that the phosphate charges and the base pair dipoles are not screened in the same manner.

The presence of PP groups can be considered as a perturbation which reduces the twisting powers of DNA molecules. In fact pyrophosphate DNA molecules described in the cited literature were short oligonucleotides while much longer DNA may be needed to study the cholesteric phase. It is not clear yet whether a sufficiently long DNA with well-defined pyrophosphate modifications can be made. An even closer similarity of the initial and modified duplexes was so far found only in computer simulations. This similarity has not been confirmed experimentally yet, so that it cannot be considered proven. For longer sequences with several pyrophosphates, this similarity may be even more questionable.

Our calculations rely on a model that is based on general and qualitative assumptions of the helical charge distribution of DNA. We feel that it accommodates a picture of DNA, without going into finer details, and agrees with the approach of paper [10], in which the continuous approximation plays a directive part. The choice of the pair potential for the intramolecular interaction is important. The shape of the pair potential chosen in this paper enabled us to accommodate the two different sets of charges —the Coulomb and the dipole ones, and also take into account a finer detail of the pyrophosphate charges, which could turn out to be a valuable instrument for further investigating the cholesteric phases. The symmetry constraints have played an important part in finding the minima of the pair potential. It seems that their meaning could be greater than a simple arithmetic device for simplifying calculations, and could indicate certain symmetry law peculiar to the cholesteric phases of DNA. It is reasonable to expect polymorphism of liquid crystalline phases of DNA. New artificially synthesized phases of DNA could be a fruitful instrument to that end.

Many of the points made above can be found in the literature, and approaches similar to our paper have been made by other authors (see, *e.g.*, the review paper [20] and references therein). However, some important differences to our work should be noted. We focus our study (and investigate in some details) on the tiny interplay between discrete charges and helically oriented also discrete dipole moments aiming to find the symmetry of the pairwise interaction energy minima.

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