ISSN 0021-3640, JETP Letters, 2012, Vol. 95, No. 1, pp. 51–54. © Pleiades Publishing, Inc., 2012.
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Orientational Order and Evolution of the Properties of Biomolecules in Anisotropic Media

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Received December 1, 2011

Experimental values of the Lorentz tensor components and the local field of a light wave have been obtained for anisotropic films of polypeptide and DNA. These data indicate an increase in the mean value and anisotropy of the polarizability of biomolecules with respect to an isotropic solution. These changes in the polarizability as an indicator of the response of biomolecules to external actions, as well as their chemical and biological activity, specify the direction of the evolution of the properties of biomolecules in orientationally ordered media.

DOI: 10.1134/S002136401201002X

1. Condensed phases of biomolecules (lipids, polypeptides, proteins, and nucleic acids) in living systems have structures similar to nematic, cholesteric, and smectic A phases of calamitic liquid crystals [1–3] and columnar phases $\mathrm{Col}_{h(o,\,d)}$ of discotic liquid crystals [4]. Since a common feature of these structures is their orientational ordering, there are questions concerning the role of the orientational order of biomolecules in the evolution of their properties in anisotropic media, physical manifestations of this evolution, and its direction.

In an anisotropic medium with a quite closed packing of molecules, the short- and long-range intermolecular interactions affect the molecular susceptibilities of various ranks, which characterize the response of molecules to external actions and adaptation of molecules to a varying environment. The lowest-rank susceptibility is the molecular polarizability tensor γ. Change in γ in an anisotropic medium reflects change in the intermolecular interactions, polarization, electronic properties, and conformation of molecules [5, 6], which affects the chemical and biological activities of molecules and their functional properties [7]. To answer the listed questions, information on change in the tensor γ of biomolecules in anisotropic media is necessary.

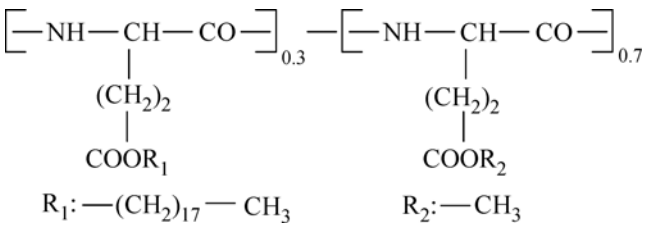
A direct method for determining the components of the tensor γ for uniaxial molecular media is refractometry, which is based on the relation $\varepsilon_j = 1 + 4\pi N f_j \gamma_j$ [5] between the components of the relative permittivity and ensemble-averaged polarizability components γ_j for light waves polarized along ($j = \|$) and normally ($j = \perp$) to the optical axis of the medium n. In this relation, N is the number of molecules per unit volume, $f_j = 1 + L_j(\varepsilon_j - 1)$ are the components of the local field tensor, and L_j are the Lorentz tensor components ($\mathrm{Tr}L = 1$). In the transparency range, $\varepsilon_j = n_j^2$, where n_j is the refractive indices of the medium. For statistically uniaxial macromolecules with the longitudinal axis **l**, the tensor γ is characterized by the longitudinal (γ_l) and transverse (γ_t) components or by the mean value $\bar{\gamma} = (\gamma_l + 2\gamma_t)/3$ and anisotropy $\Delta\gamma = \gamma_l - \gamma_t$. For biomolecules in anisotropic media, $\bar{\gamma}$ and $\Delta\gamma$ were determined using the quantity [8, 9]

$$\bar{\gamma}_H = \frac{3(\bar{\varepsilon} - 1)}{4\pi N(\bar{\varepsilon} + 2)} \qquad (1)$$

for $\bar{\gamma}$ (where $\bar{\varepsilon} = (\varepsilon_\| + 2\varepsilon_\perp)/3$), isotropic tensor $f_V = (\bar{\varepsilon} + 2)/3$ [8, 9], or components $L_j = 1/3$ [10, 11]. These models predetermine $\bar{\gamma}$ and $\Delta\gamma$ values and are responsible for unphysical consequences from spectral data [5].

Objective study of changes in $\bar{\gamma}$ and $\Delta\gamma$ for biomolecules in uniaxial media is possible when the components L_j [12] are determined from experimental data without a priori assumptions on unobservable parameters of molecules and properties of tensors L and f. This approach is used here for polypeptide biomolecules and DNA in uniaxial films to reveal constraints imposed on $\bar{\gamma}$ and $\Delta\gamma$ values by the formation of a film and intermolecular interactions. These constraints provide answers to the questions formulated above.

2. Let us consider a uniaxial film with the planar (parallel to the film plane) or homeotropic (perpendicular to the film) orientation of the optical axis **n**. Let the film consist of statistically uniaxial macromolecules whose orientational order with respect to **n** is



Structure formula of the polyglutamate monomer.

characterized by the value $S = \langle 3\cos^2\theta - 1\rangle/2$, where θ is the angle between axes **l** and **n** and triangular brackets means average over the ensemble of macromolecules. The sign of S determines the axial ($0 < S \le 1$) or planar ($-0.5 \le S < 0$) orientation of macromolecules. The procedure of determining components $L_\perp$ and $L_\parallel = 1 - L_\perp$ depends on the sign of $\Delta n = (n_\parallel - n_\perp) \sim \Delta\gamma S$ [12].

For films studied here with $\Delta n > 0$ in the visible transparency range, the parameters $\bar\varepsilon$ and $Q = (\varepsilon_\parallel - \varepsilon_\perp)/(\bar\varepsilon - 1)$ are used along with the quantities

$$r_0 = 1 - \frac{2Q^2(\bar\varepsilon - 1)}{3(3+Q)(\bar\varepsilon + 2)}, \quad b = \frac{3(\bar\varepsilon - 1)}{4\pi N\bar\gamma(\bar\varepsilon + 2)} - r_0, \tag{2}$$
$$b_1 = \frac{2r_0Q^2}{(3-Q)(3+2Q)}, \quad b_2 = b_1[(6+Q)/Q]^2.$$

For the film state characterized by index T, these quantities are functions of T and the wavelength of light λ. The desired $L_\perp(T)$ value is given by the expression [12]

$$L_\perp = L_{\perp k} - \frac{(\bar\varepsilon + 2)}{12(\bar\varepsilon - 1)} \times [(b_1b_2)^{1/2} - b - [(b_1 - b)(b_2 - b)]^{1/2}], \tag{3}$$

where $L_{\perp k} = (3 + 2Q)/[3(3 + Q)]$ and the function $b(\lambda, T)$ depends on the unknown function $\bar\gamma(\lambda, T)$. The component $L_\perp(T)$ is determined as follows. At known $n_j(\lambda, T)$ values and for a discrete set of λ_i ($i = 1 - p$) in the visible range, the function $b(\lambda, T)$ in the interval λ_1–λ_p is approximated by the polynomial

$$b(\lambda, T) = a_0(T) + a_1(T)\lambda + \ldots + a_m(T)\lambda^m. \tag{4}$$

The quantity $L_\perp(T)$ is independent of λ and the state T corresponds to $m + 2$ unknowns ($L_\perp^{(m)}$, $a_0 - a_m$). They are determined from the system of $m + 2 = p$ equations (3) each corresponding to one of the λ_i values. The criterion of the adequacy of the approximation used in Eq. (4) is the agreement of the $L_\perp^{(m)}$ values with the $\langle L_\perp^{(m-1)}\rangle$ values averaged over the $L_\perp^{(m-1)}$ values corresponding to combinations of $p - 1$ reference wavelengths λ_i from the set (λ_1, λ_2, ..., λ_p) [12].

3. Polyglutamate polypeptide copolymer includes α-helical rod-like macromolecules with the structure of monomers shown in the figure [13].

For a uniaxial nematic polyglutamate film with a thickness of 0.5425 μm with the planar orientation of n and the axial orientation of molecular axes **l** of α helixes with respect to **n**, the refractive indices $n_j(\lambda_i)$ are tabulated in [13] with an accuracy of 10^{-4} at values $\lambda_1 = 0.4762$ μm, $\lambda_2 = 0.4825$ μm, $\lambda_3 = 0.5309$ μm, $\lambda_4 = 0.5682$ μm, and $\lambda_5 = 0.6471$ μm in the transparency range. The side fragments of polyglutamate monomers are orientationally disordered and do not noticeably contribute to Δn. As a result, for polyglutamate molecules in the film, $\Delta\gamma > 0$, $S > 0$, and $\Delta n > 0$. The use of the $n_j(\lambda_i)$ values [13] for the polyglutamate film gives the same values $L_\perp^{(3)} = 0.3412$ and $\langle L_\perp^{(2)}\rangle = 0.3410 \pm 0.0004$.

Uniaxial $DNA_{1,2}$ films 2–4 μm in thickness with the homeotropic orientation of **n** and the planar orientation of rod-like helical DNA macromolecules of natural sea salmon [14] were also studied. The DNA_1 (DNA_2) films were dried in vacuum at 35–45°C (they were stored in air at 21°C) for a day. Their refractive indices $n_j(\lambda_i)$ measured at a relative humidity of 45% (50–55%) are tabulated in [14] with an accuracy of 10^{-4} at $\lambda_1 = 0.6328$ μm and $\lambda_2 = 0.8140$ μm in the transparency range. The polarizability anisotropy $\Delta\gamma_m$ of the monomer fragment of DNA and the quantity $\Delta\gamma$ for the macromolecule satisfy the inequalities $\Delta\gamma_m < 0$ and $\Delta\gamma < 0$ [15]; correspondingly, $\Delta n > 0$ at $S < 0$ for $DNA_{1,2}$. The $n_j(\lambda_i)$ values give close values $L_\perp^{(0)} = 0.3421$ and 0.3407 for the DNA_1 and DNA_2 films, respectively.

The parameters $\bar f = (f_\parallel + 2f_\perp)/3$ and $\Delta f = f_\parallel - f_\perp$ can be represented in the form

$$\bar f = (\bar\varepsilon + 2)[1 - A(1 - r_0)]/3, \quad \Delta f = Q(\bar\varepsilon - 1)(1 - A)/3, \tag{5}$$

where $A = (L_\perp - 1/3)/(L_{\perp k} - 1/3)$. For polyglutamate and $DNA_{1,2}$ films, the resulting $L_\perp$ values in the transparency range correspond to the inequalities $L_\perp > L_{\perp k} > 1/3$, $A > 1$, and $\Delta f < 0$. The inequality $S\Delta\gamma\Delta f < 0$ for these objects in the relation

$$\bar\varepsilon - 1 = 4\pi N(\bar\gamma\bar f + 2S\Delta\gamma\Delta f/9) \tag{6}$$

leads to the constraints

$$\bar\gamma > \frac{\bar\varepsilon - 1}{4\pi N\bar f} > \frac{3(\bar\varepsilon - 1)}{4\pi N r_0(\bar\varepsilon + 2)} \equiv \bar\gamma_0. \tag{7}$$

The quantity $S\Delta\gamma$ is given by the expression [12]

$$S\Delta\gamma = \bar{\gamma}Q(1+\sigma), \quad (8)$$

where the correction σ to anisotropy Δf has the form

$$\sigma = \frac{\Delta f(Q^2-9)(3+2Q)}{Q[3(3+Q)(\bar{\varepsilon}+2)r_0 + \Delta f(3-Q)(3+2Q)]}. \quad (9)$$

For the discussed objects, the quantities $\sigma > 0$ are not small and are significant for determining $S\Delta\gamma$ values from Eq. (8). For polyglutamate, $\sigma(\lambda_5) = 0.652$; for DNA_1 (DNA_2), $\sigma(\lambda_1) = 0.305$ (0.245). The constraints

$$S\Delta\gamma > \frac{\Delta\varepsilon(1+\sigma)}{4\pi N\bar{f}} > \frac{3\Delta\varepsilon}{4\pi N r_0(\bar{\varepsilon}+2)} \equiv S\Delta\gamma_0 \quad (10)$$

follow from Eqs. (7) and (8). The upper estimates for $\bar{\gamma}$ and $S\Delta\gamma$ in Eqs. (7) and (10) are close to their exact values in Eqs. (6) and (8). The right-hand sides of Eqs. (7) and (10) correspond to the $\bar{\gamma}_0$ and $S\Delta\gamma_0$ values at $\Delta f = 0$ and the isotropic tensor $f = \bar{f}$ ($A = 1$) $= r_0(\bar{\varepsilon} + 2)/3$.

At given Q and S values determined by the film preparation conditions, constraints (7) and (10) reflect the effect of intermolecular interactions in the film on the polarizability components

$$\gamma_l = \bar{\gamma}\left[1 + \frac{2Q(1+\sigma)}{3S}\right], \quad \gamma_t = \bar{\gamma}\left[1 - \frac{Q(1+\sigma)}{3S}\right], \quad (11)$$

which change consistently with the quantities $n_j(\lambda)$, L_j, and $f_j(\lambda)$. For the polyglutamate film with $Q > 0$ and $S > 0$, the experimental value $\sigma > 0$ increases (reduces) the quantities γ_l and $\Delta\gamma(\gamma_t)$ with respect to their magnitudes corresponding to the models with $\sigma \le 0$. The same is valid for lipid molecules in a bilayer lipid membrane and smectics A [12], as well as for molecules with $\Delta\gamma > 0$ in a quasinematic layer of cholesteric liquid crystals [16].

For the $DNA_{1,2}$ films with $Q > 0$ and $S < 0$, the experimental values $\sigma > 0$ reduce (increase) the quantities γ_l (γ_t and $|\Delta\gamma|$) with respect to their magnitudes corresponding to the models with $\sigma \le 0$. The same should be expected for quasinematic layers of cholesteric dispersions of DNA [3] and the columnar hexagonal phase of DNA [4], which is similar to the $Col_{h(o,d)}$ phases of discotic liquid crystals with $Q < 0$, $S > 0$, and $\sigma > 0$ [12].

Thus, for all known orientationally ordered condensed phases of biomolecules with the structure of calamitic or discotic liquid crystals, the experimental L_j and f_j values obtained without a priori assumptions on unobservable molecular parameters and properties of the tensors L and f correspond to lower bounds (7) and (10) of the parameters $\bar{\gamma}$ and $\Delta\gamma$ (or $|\Delta\gamma|$).

4. Let us compare the result with the data of the model approaches for determining $\bar{\gamma}$ and $\Delta\gamma$ in anisotropic biomolecular media. According to Eqs. (1) and (7), it follows from the inequality $r_0 < 1$ that $\bar{\gamma}_0 > \bar{\gamma}_H$ and $\bar{\gamma}_H$ is smaller than $\bar{\gamma}$. The expression for b in Eqs. (2) indicates that the condition $\bar{\gamma} = \bar{\gamma}_H$ [8, 9] corresponds to the quantity $b(\bar{\gamma}_H) = 1 - r_0 = b_H > 0$ and values $L_\perp(b_H) = L_\perp^{(H)} < L_{\perp k}$ and $\Delta f(L_\perp^{(H)}) > 0$. Now, Eq. (8) changes to

$$S\Delta\gamma_H = \bar{\gamma}_H Q(1+\sigma_H) \quad (12)$$

with $\sigma_H < 0$. For polyglutamate, $\sigma_H(\lambda_5) = -0.159$; for DNA_1 (DNA_2), $\sigma_H(\lambda_1) = -0.177$ (-0.170). It follows from Eqs. (8) and (12) that

$$\Delta\gamma_H/\Delta\gamma = \bar{\gamma}_H(1+\sigma_H)/[\bar{\gamma}(1+\sigma)] \quad (13)$$

and $\Delta\gamma_H$ is much smaller than $\Delta\gamma$.

The use of the tensor $f_V = (\bar{\varepsilon} + 2)/3$ [8, 9] with $\Delta f_V = \sigma_V = 0$ in Eqs. (6), (8), and (10) gives $\bar{\gamma}_V = \bar{\gamma}_H$ and the value $\Delta\gamma_V = \Delta\gamma\bar{\gamma}_H/[\bar{\gamma}(1+\sigma)] = \Delta\gamma_0 r_0$ smaller than $\Delta\gamma_0$.

The values $L_j = 1/3$ [10, 11] correspond to $A = 0$, $\bar{f}^* = (\bar{\varepsilon} + 2)/3$, $\Delta f^* = Q(\bar{\varepsilon} - 1)/3$, and

$$S\Delta\gamma^* = \bar{\gamma}^* Q(1+\sigma^*) \quad (14)$$

with $\sigma^* < 0$. With allowance for inequality $S\Delta\gamma^*\Delta f^* > 0$, the constraint $\bar{\gamma}^* < \bar{\gamma}_H$ follows from Eq. (6). For polyglutamate, $\sigma^*(\lambda_5) = -0.294$; for DNA_1 (DNA_2), $\sigma^*(\lambda_1) = -0.324$ (-0.313); i.e., the $|\sigma^*|$ values are larger than the $|\sigma_H|$ values by almost a factor of 2. In the limit $Q \longrightarrow 0$,

$$\sigma^*_{\lim} = (1-\bar{\varepsilon})/(\bar{\varepsilon}+2), \quad (15)$$

and $1 + \sigma^* < 1 + \sigma^*_{\lim} = 3/(\bar{\varepsilon} + 2)$.

Let us compare the $\Delta\gamma^*$ value for rigid chain macromolecules in the anisotropic film with the $\Delta\gamma_s$ value for the same macromolecules in the solution. It is known that the $\Delta\gamma_s$ values for rigid anisotropic molecules are close to the $\Delta\gamma_V$ values obtained for these molecules from the refractive indices of anisotropic media using the tensor f_V [17]. Taking into account this circumstance and inequality $\bar{\gamma}^* < \bar{\gamma}_H$, we obtain

$$\Delta\gamma^*/\Delta\gamma_s \approx \Delta\gamma^*/\Delta\gamma_V < 1 + \sigma^* < 3/(\bar{\varepsilon}+2). \quad (16)$$

For uniaxial DNA films (*calf thymus* DNA) with the homeotropic orientation of **n** and the planar orientation of molecules studied in [11], the average refractive index $n = 1.47$ ($\lambda = 0.55$ μm) provides the constraint $\Delta\gamma^*/\Delta\gamma_s < 0.721 = 3/(\varepsilon + 2)$. This upper estimate is in agreement with the experimental value $\Delta\gamma^*/\Delta\gamma_s \approx 0.61$ [11] and explains its value and cause. With the σ^* val-

ues presented above, for the DNA_1 (DNA_2) film at $\lambda = \lambda_1$, it follows from Eq. (16) that $\Delta\gamma^*/\Delta\gamma_s < 0.676$ ($\Delta\gamma^*/\Delta\gamma_s < 0.687$) in agreement with the data from [11]. For the polyglutamate film at $\lambda = \lambda_5$, $\Delta\gamma^*/\Delta\gamma_s < 0.706$.

5. The summary of the above consideration can be given in the form of the inequalities

$$\begin{aligned} \bar{\gamma}_0 > \bar{\gamma}_H = \bar{\gamma}_V \approx \bar{\gamma}_s > \bar{\gamma}^*, \\ \Delta\gamma_0 > \Delta\gamma_s \approx \Delta\gamma_V > \Delta\gamma_H > \Delta\gamma^*, \end{aligned} \quad (17)$$

which, together with constraints (7) and (10), answer the formulated questions. An increase in the $\bar{\gamma}$ and $\Delta\gamma$ values in anisotropic biomolecular media specifies the direction of the evolution of physicochemical, biological, and functional properties of molecules, which depend on $\bar{\gamma}$ and $\Delta\gamma$. For any conditions of the formation of a natural or artificial anisotropic molecular medium, the presence of the long-range orientational order of molecules in it and the anisotropy of the short-range coordination environment of molecules determines anisotropic intermolecular interactions, which are manifested in the anisotropy of the tensors L and f [18] and in change in the molecular parameters $\bar{\gamma}$ and $\Delta\gamma$.

For this reason, anisotropic biomolecular media are promising for biological evolution of biomolecules against the background of isotropic condensed media and solutions, because the physical and biochemical processes responsible for an increase in $\bar{\gamma}$ and $\Delta\gamma$ in an anisotropic biomolecular ensemble increase its stability, which, in turn, promotes the occurrence of these processes. Thus, owing to the orientational order of fairly close packed biomolecules, chemical reactions and processes promoting an increase in $\bar{\gamma}$ and $\Delta\gamma$ are autocatalytic. In particular, the process of renaturation of DNA molecules with a decrease in the temperature is more efficient when they are in quasinematic layers of cholesteric dispersions [3] than in the case of isolated DNA molecules in the solution, because it is accompanied by an increase in $\bar{\gamma}$ and $\Delta\gamma$, along with the steric constraints from neighboring molecules [3].

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Translated by R. Tyapaev