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THE EFFECT OF CONSTANT MAGNETIC AND LIGHT-WAVE FIELDS ON THE LUMINESCENCE POLARIZATION DEGREE IN AN AQUEOUS GLYCYLTRYPTOPHAN SOLUTION

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The effect of external fields on the luminescence of the (0.1-1) mg ml^{-1} aqueous glycyltryptophan solution was studied. It was found that the solution "memorizes" the prolonged external action of both a constant magnetic field with induction of 0.1 T and a linearly polarized quasimonochromatic light-wave field with intensity of about 1 W m^{-2}. On termination of the exposure, the relaxation processes had a nonmonotonic behavior of a cyclic character with characteristic times of the order of a few tens of minutes. The results obtained were interpreted in terms of the concept of an interrelation between structural rearrangements in water and conformational variations of solute molecules.

To date, experimental results are available that indicate that magnetic [1] and light-wave [1, 2] fields affect the properties of biological systems. However, there is no clearly defined physical model that could adequately explain the mechanism of action of electromagnetic fields. Studying, at the molecular level, the effect of magnetic and light-wave fields on the properties of biological systems could contribute to the development of such a physical model.

The purpose of this work was to study the nature of variations in the luminescence parameters of model biological systems exposed to constant magnetic (CM) and light-wave (LW) fields, as well as the kinetics of these variations after termination of exposure.

We have chosen as a model system an aqueous glycyltryptophan solution whose molecule can be thought of as a fragment of a polypeptide chain from which a protein globule forms. A natural marker by which the molecule conformational variations could be followed was the indole ring of the aminoacid tryptophan residue, which produces intensive luminescence in the UV region of the spectrum and is uniquely sensitive to the environment.

We studied the absorption luminescence polarization spectrum, which is highly sensitive to conformational rearrangements of both the molecule itself and the ensemble of bonded molecules, to any variations in the hydrate (solvate) shell of molecules, and to orientational and translational motions of molecules [3]. In our experiment, the excitation wavelength λ_{ex} was varied from 250 to 310 nm; the emission wavelength corresponded to the maximum intensity of the luminescence spectrum, $\lambda_{em\ max}$ = 360 nm. The relative measurement error for the polarization degree was 5-10%.

A neutral aqueous glycyltryptophan solution at room temperature (20°C) and pH = 5.7 was poured into quartz cells of two types: rectangular cells 10 × 10 × 40 mm in size and ring-shaped cells 24 mm in diameter and 1 mm deep. The differently shaped cells were used to make sure that convection flows do not affect the effect studied.

The luminescence spectra were measured on a Hitachi MPF-4 spectrofluorimeter. The cells were placed in the fluorimeter chamber and exposed to linearly polarized light, which was directed normally to the rectangular cells and frontally to the ring-shaped cells [4]. The spectrofluorimeter was coupled with an RPT-80 microcomputer. The measured luminescence absorption spectra $I(\lambda_{ex})$ were recorded on a plotter and simultaneously fed to the microcomputer storage to be subsequently processed and polarization absorption spectra were calculated at 1-nm spacing. The measured luminescence spectra and the calculated polarization spectra were simultaneously displayed alphanumerically and graphically. The polarization degree P (in

percent) was calculated by the formula [3, 4]

$$P = \frac{1 - AI_{vh}/I_{vv}}{1 + AI_{vh}/I_{vv}} \cdot 100\%, \quad (1)$$

where $A = I_{hv}/I_{hh}$ is the correcting factor allowing for the device polarization effect; I_{vv} is the recorded signal intensity for vertical orientation of the input and output polarizers in the spectrofluorimeter chamber; I_{vh} is the signal intensity for vertical orientation of the input polarizer and horizontal orientation of the output polarizer; I_{hh} is the signal intensity for horizontal orientation of the input and output polarizers; I_{hv} is the signal intensity for the horizontally oriented input polarizer and the vertically oriented output polarizer. The recording time of the four spectra, I_{vh}, I_{vv}, I_{hv}, and I_{hh}, was between 5 and 10 min. In order to determine the time behavior of the luminescence $I(\lambda_{ex})$ and the polarization $P(\lambda_{ex})$ spectra, the above-mentioned combinations of the spectra were recorded in succession at an interval of a few minutes during an hour or for a longer time period. This procedure of measuring and processing the results made possible visual observation of the variation of luminescence parameters in time. In the course of measurements, the solution temperature was monitored with the help of a thermocouple.

The described method was applied to determine the polarization spectra $P(\lambda_{ex})$ for different concentrations C of glycyltryptophan in water over the range of $C = (5 \times 10^{-2} - 1)$ mg/ml. For each concentration, $P(\lambda_{ex})$ was determined for two identical cells, one of which, in subsequent experiments, served as the reference cell (not exposed to external fields). To eliminate a possible effect of the light-wave field under conditions of natural lighting, the reference cell was placed in a light-proof chamber. Two types of external field action on the solution in the working cell were used. The first was exposure to a linearly polarized light with an intensity of 1 W m^{-2} and a wavelength of 550 ± 10 nm, in the region at a distance from the glycyltryptophan absorption band, during a time interval τ_{inf}. In different experiments, τ_{inf} was varied from a few minutes to several hours. The second type was exposure to a constant uniform magnetic field with induction of 10^{-1} T (the strength $H = 10^3$ Oe) produced in the constant magnet gap; τ_{inf} was varied in the same range. To eliminate the superposition of a possible effect of the natural light-wave field on the magnetic field action, the cell was placed into a light-proof casing. The relative variation of the solution temperature and its pH did not exceed 5% during the exposure time τ_{inf}.

Immediately after termination of the exposure, the luminescence spectra were recorded and processed using the procedure described above. Concurrently, measurements were performed in the reference cell which had not been exposed to an external action. The changes observed in the reference cell were inconsistent and exceeded but slightly the measurement error.

It has been found in numerous experiments that exposure to both the CM field and the LW field results in an increase in the luminescence polarization degree over the entire absorption band. The intensity of luminescence varies while the position of its maximum in the luminescence and polarization spectra remains the same. The variation of the polarization degree is "memorized" by the solution for a few tens of minutes after exposure termination. Figure 1 depicts the polarization spectra $P(\lambda_{ex})$ prior to and directly after the exposure to a linearly polarized LW field. Each curve represents P values averaged over the time of recording of the four spectra. Curve 1 corresponding to $P(\lambda_{ex})$ prior to the exposure has a shape typical of compounds containing an indole ring: with a typical "dip" in the 265-295 nm wavelength range and an "ascent" at the absorption band edges, which is especially sharp in the long-wave region [5]. As shown by curve 1, the absolute value of P is maximum at $\lambda_{ex} = 310$ nm and equals 5.2%, while in the middle of the absorption band it drops to 1.4%. The relatively low values of P over the entire absorption band are due to the low viscosity of water and correlate with the literature data on the dependence of the luminescence polarization degree on the type of solvent in compounds containing an indole ring [5]. The appreciable nonuniformity of the polarization spectrum by the absorption band correlates well with a model of two mutually perpendicular linear oscillators of the indole ring, whose contributions to absorption and emission are different for different regions of the absorption band and the ratio of these contributions is determined by the environment (the medium polarity in particular) [3, 5]. Curve 2 in Fig. 1 illustrates $P(\lambda_{ex})$ immediately after the exposure to linearly polarized light with a 550 ± 10 nm wavelength and $\tau_{inf} = 20$ min. Curve 2 shows that P increases over the entire absorption band without any noticeable change in the form of the polarization spectrum. The maximum increase of P (by about 2.5 times) is observed in the middle of the absorption band. The curves in Fig. 1 refer to a solution with a concentration $C = 0.1$ mg/ml. For solutions with $C = 0.05$, 0.5, and 1.0 mg/ml, $P(\lambda_{ex})$ was also observed to grow but the effect was less pronounced; therefore most

measurements were taken for $C = 0.1$ mg/ml. The concentration dependence has not been studied in much detail.

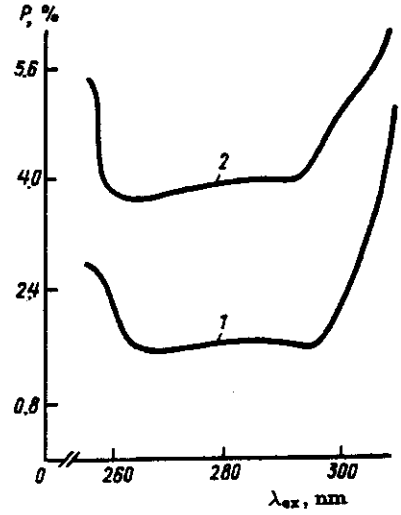

Fig. 1

Polarization absorption spectrum of the 0.1 mg/ml aqueous glycyltryptophan solution before (1) and immediately after (2) exposure to a light-wave field.

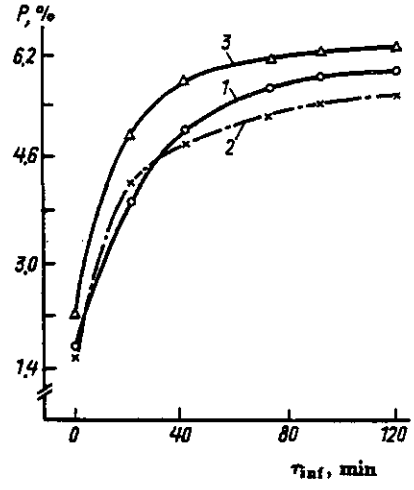

Fig. 2

Luminescence polarization degree versus time of exposure to light-wave field at (1) $\lambda_{ex} = 280$ nm, (2) $\lambda_{ex} = 290$ nm, and (3) $\lambda_{ex} = 300$ nm.

The results of studying the $P(\lambda_{ex})$ buildup as a function of time of exposure to the LW field are illustrated in Fig. 2. Curves 1, 2, and 3 refer to different λ_{ex}. The value of P at $\tau_{inf} = 0$ corresponds to the polarization degree prior to exposure. As is seen in Fig. 2, an increase in τ_{inf} from 20 to 40 min causes P to grow sharply for any λ_{ex}. A further increase in τ_{inf} slows down the growth of P, and $P(\lambda_{ex})$ attains saturation at $\tau_{inf} \gtrsim 90$ min.

Studying the behavior of $P(\lambda_{ex})$ after termination of the exposure to the LW field revealed a nonmonotonic course of the relaxation processes: within characteristic time intervals of the order of a few tens of minutes, $P(\lambda_{ex})$ first dropped to the values equal to or less than the values of $P(\lambda_{ex})$ prior to the exposure and then grew up to the level corresponding to the polarization degree immediately after the exposure terminated. This quasicyclic character of relaxation process is illustrated in Fig. 3, where typical $P(t)$ curves are given for different λ_{ex}. In Fig. 3, $t = 0$ corresponds to P just before the exposure initiation, and $t = 20$ min to P immediately after the end of the exposure. As is seen in the figure, the time variation of P can be described in terms of time intervals $\tau_1 = 110$ min and $\tau_2 = 20$ min which characterize, respectively, the time of reaching the minimum and the maximum value of P. The total cycle time $\tau_c = \tau_1 + \tau_2 = 130$ min. Figure 3 demonstrates that τ_1 is several fold greater than τ_2 and τ_2 is comparable to the exposure duration $\tau_{inf} = 20$ min.

As demonstrated by additional experiments, the effect described is caused exclusively by the field of a linearly polarized light wave at a distance from the glycyltryptophan absorption band. This effect has not been observed in solutions exposed to unpolarized light with the same intensity and the same exposure time τ_{inf}, nor in solutions exposed to 280-nm linearly polarized light, i.e., at the glycyltryptophan absorption maximum (of the same intensity and duration τ_{inf}).

When the glycyltryptophan solution with $C = 0.1$ mg/ml was exposed to a CM field with induction of 0.1 T ($H = 10^3$ Oe), $P(\lambda_{ex})$ grew in a similar manner over the entire absorption band followed by a typical quasicyclic relaxation process. However, in this case, a noticeable effect could be obtained by increasing τ_{inf} several fold. Figure 4 depicts a typical course of the relaxation process induced in a solution exposed to the CM field for $\tau_{inf} = 120$ min. In the figure, $t = 0$ corresponds to P prior to the exposure and $t = 120$ min to P immediately after the exposure termination. In the same manner as in Fig. 3, one can determine the characteristic time intervals $\tau_1 = 80$ min and $\tau_2 = 50$ min. One can see that the course of the relaxation process differs somewhat from relaxation occurring after the end of an LW-field exposure: the minimum of P does not exceed its initial value (at $t = 0$); τ_1 and τ_2 become comparable and $\tau_2 < \tau_{inf}$. However, the total cycle time $\tau_c = \tau_1 + \tau_2 = 130$ min remains the same.

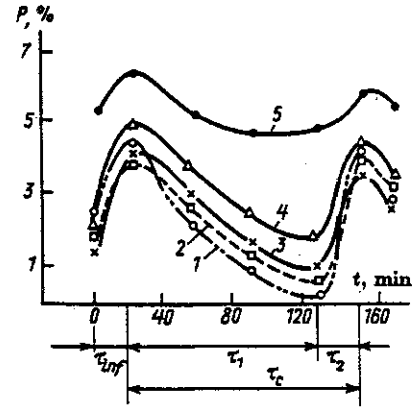

Fig. 3

Luminescence polarization degree versus time elapsed after termination of exposure to a light-wave field with (1) $\lambda_{ex} = 260$ nm; (2) $\lambda_{ex} = 280$ nm; (3) $\lambda_{ex} = 290$ nm; (4) $\lambda_{ex} = 300$ nm; and (5) $\lambda_{ex} = 310$ nm.

The observed increase in $P(\lambda_{ex})$, which results from the solution exposure to the LW field and the CM field, indicates an improvement of the orientational ordering of glycyltryptophan molecules.

The slow nonmonotonic time variation of $P(\lambda_{ex})$ (after termination of the solution exposure to the LW or the CM field) with characteristic times of the order of tens of minutes can be considered as the effect of "memorizing" a prolonged external action by the solution.

The described effects can be interpreted in terms of the concept of an orienting action of an external field

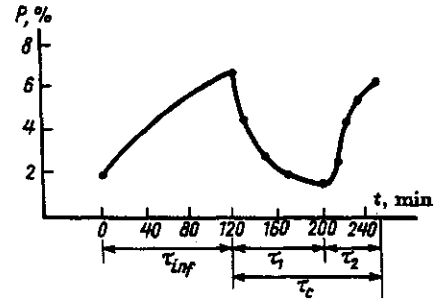

Fig. 4

Luminescence polarization degree versus time elapsed after termination of exposure to a constant magnetic field with $\lambda_{ex} = 280$ nm.

on solute molecules, which leads to interrelated conformational rearrangements of the compound molecules and to structural rearrangements of the solvent (water). Complex molecules, in particular molecules containing cyclic elements, may possess considerable anisotropy of the magnetic, $\Delta\chi$, and dielectric, $\Delta\alpha$, susceptibilities, and also have a nonzero intrinsic magnetic moment p_m. Such is the glycyltryptophan molecule containing two cyclic elements. In a constant magnetic field with strength H, the molecule is exposed to torques equal to $p_m H$ and $(1/2)(\Delta\chi)H^2$ in magnitude. In a linearly polarized light field, the molecule is exposed to both a magnetic and an electric field. Since these fields are variable, the orienting action is quadratic. The torques produced are equal to $1/2(\Delta\chi)H_l^2$ and $1/2(\Delta\alpha)E_l^2$, where H_l and E_l are, respectively, the intensity of the electric and the magnetic field in the light wave. Under our experimental conditions, the torque for a single molecule is considerably lower than the energy kT of thermal motion; therefore the external field orienting action can manifest itself only for an ensemble of cooperatively interacting molecules bonded by dispersion forces. For the energy of orientational motions to be greater than kT, such an ensemble should contain at least one hundred molecules. The ensemble's orientational motions are accompanied with a rearrangement of the solvate shell and with the transition of molecules to new conformations that would correspond to an energetically stable state in the external field. The setting time for the new conformational state can be assessed by applying to the ensemble of molecules the method of calculation used in [6] for the protein molecule. For an ensemble of molecules to pass to a new conformational state, it must have stored enough energy to overcome the activation barrier. To estimate the characteristic time of energy accumulation we use the following relation:

$$\tau = \tau_* \exp\left\{\frac{\Delta G}{kT}\right\}, \tag{2}$$

where ΔG is the height of the activation barrier; τ_* is the mean time needed for the ensemble of molecules to rotate by an angle of 180° in Brownian motion. Since $P(\lambda_{ex}) > 0$ prior to exposure to the external field, we can assume that $\tau_* \gtrsim \tau_{inf}$, where $\tau_{inf} \simeq 10^{-8}$ s is the time of molecule excited state. It is reasonable to assign to ΔG a value of the order of dispersion interaction energy, that is, $\Delta G \simeq 0.6$-0.65 eV. Since at room temperature $kT = 2.5 \times 10^{-2}$ eV, we get $\tau \simeq 4.5$-33 min according to relation (2). As Fig. 2 demonstrates, the experimentally-found characteristic time in which P attains saturation (and the molecules in the solution become orientationally ordered) is about 40 min, which agrees with the theoretically estimated value of τ. In line with our discussion, one should expect that once the external field is removed the ensemble of molecules returns to the initial state within a time comparable to τ as assessed by formula (2). One can see from Figs. 3 and 4 that this relaxation does occur within 40-80 min, which is comparable to τ. The subsequent quasicyclic character of $P(t)$ can be understood if the dynamics of structural rearrangements in the solvent (water) is taken into account. According to current concepts [7, 8], pure water features a definite topology of probable structures with an invariable transition dynamics ("flickering clusters"). The compound molecules introduced into water disrupt the structure topology and affect the "flickering cluster" dynamics. Both in pure water and in solutions, structural transitions can [9] be quasiperiodic with an assumed period between tens of seconds and tens of minutes. Clearly, conformational changes in a molecular ensemble will also lead to structural perturbations that will affect the period and amplitude of quasiperiodic structural transitions in

water. According to the ideas developed in [10], the modified dynamics of structural transitions may act back on the conformational state of solute molecules and give rise to quasiperiodic conformational oscillations. It seems that the quasicyclic time variation of P observed in this work reflects the relationship between the conformational transitions in the ensemble of solute molecules and the structural rearrangements in water induced by external fields.

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