Light-induced diffusion of DNA in solutions due to laser photomodification

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A new nonlinear optical effect, in which DNA molecules are ejected from the laser focus according to the diffusion mechanism, is predicted theoretically and observed experimentally.

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Stockmann[1] and Parkhomenko *et al.*[2] predicted and observed a new phenomenon—a nonlinear laser modification (NLM) of DNA. The long, linear DNA molecules break down into shorter sections as a result of irradiation of solutions of DNA (that were stained by a specific dye) focused, by a highly efficient laser light. This phenomenon can be explained in terms of transfer to DNA of a two-photon excitation of the dye. In this communication we discuss the influence of the NLM on the spatial distribution of the DNA density.

Let us assume that a certain region is irradiated in the DNA solution when a NLM occurs.[1,2] If NLM occurs directly as a result of irradiation,[1)] then the irradiated volume will contain split molecules which have larger values of the diffusion coefficient D than the original molecules; hence they diffuse from this volume faster than the whole molecules penetrate into it from the neighboring regions. Therefore, the (optical) density[2)] of DNA collapses in the irradiated volume and increases in the neighboring regions. We call the described effect, which is similar to the diffusive density redistribution of atoms in the gases,[3)] the light-induced diffusion of DNA (LID/DNA).

We shall examine a case in which the irradiated time τ_{ir} is much smaller than the diffusion time $\tau_D = a^2/4D$, where a is the characteristic length of the irradiated region. Thus, the potential density ρ, which is constant immediately after the irradiation, begins to vary $\Delta\rho$ after the time $t \sim \tau_D$; at $t \gg \tau_D : |\Delta\rho| \sim (\tau_D/t)^{1/2}$. During the NLM process, molecules with different lengths are formed, i.e., at each point $\mathbf{r}$ the density distribution $P(\mathbf{r},D)$ of DNA is formed according to the values of D. The solution of the

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diffusion problem has the form

$$\Delta\rho(\mathbf{r},t) = \int d^3r\, dD\, G_D(\mathbf{r}-\mathbf{r}',t)[P(\mathbf{r}',D) - \bar{P}(\mathbf{r}')\delta(D-D_0)];$$

$$G_D(\mathbf{r},t) \equiv (4\pi Dt)^{-3/2}\exp(-r^2/4Dt),\ \bar{P}(\mathbf{r}) \equiv \int dD P(\mathbf{r},D), \tag{1}$$

where D_0 is the value of D for the original DNA molecules. Let us determine $P(\mathbf{r},D)$ after a weak irradiation, when two breaks in one molecule are unlikely. By assuming realistically that under conditions[2] of NLM the breaks occur randomly along the molecule's chain and that the values of D^{-1} is approximately proportional to the molecule's length, we obtain $P(\mathbf{r},D) = 2D_0^2 D^{-3}\theta(D-D_0)\bar{P}(\mathbf{r})$. Let us examine a one-dimensional problem in which the irradiated region has the shape of a plate of width $2a$ along the x axis. Parametrizing $\bar{P}(\mathbf{r}) = \rho P_S \exp(-x^2/a^2)$, where $P_S = \text{const}$ is the fraction of molecules that are split at the center, we obtain from Eq. (1):

$$\Delta\rho(\mathbf{r},t) = \rho P_S\left[2\int_0^1 f(k)\,k\,dk - f(1)\right];$$

$$f(k) \equiv (1+t/\tau_k)^{-1/2}\exp[-(x/a)^2(1+t/\tau_k)^{-1}], \tag{2}$$

where $\tau_k \equiv a^2/4kD$. This dependence is illustrated in Fig. 1.

We performed an experiment involving the observation of LID/DNA; the experimental arrangement is illustrated in Fig. 2. The experiment involved two stages. First we performed an NLM: a solution of stained DNA in a 0.1-cm-thick (along the beam) quartz cell was irradiated by a focused light from a nitrogen laser. The concentrations and light parameters of the DNA and the dye were the same as those in Ref. 2. The focal spot, which was moved parallel to the cell plane, illuminated an area of width $2a = 60\ \mu\text{m}$ and height 0.6 cm, so that the one-dimensional conditions were satisfied.

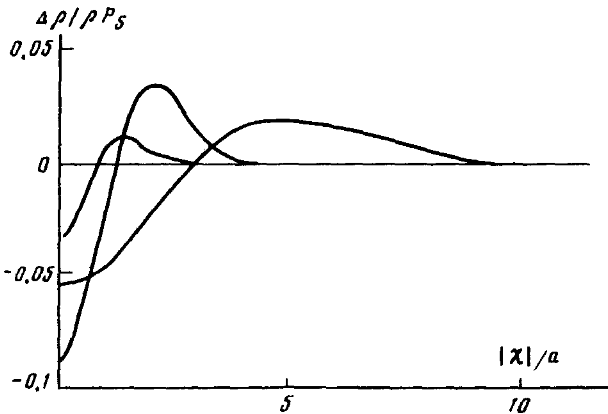

FIG. 1. Theoretical spatial dependences of the optical density. Curves 1, 2, and 3 correspond to the times $t/\tau_1 = 0.1$, 1.3 (maximum effect), and 10.

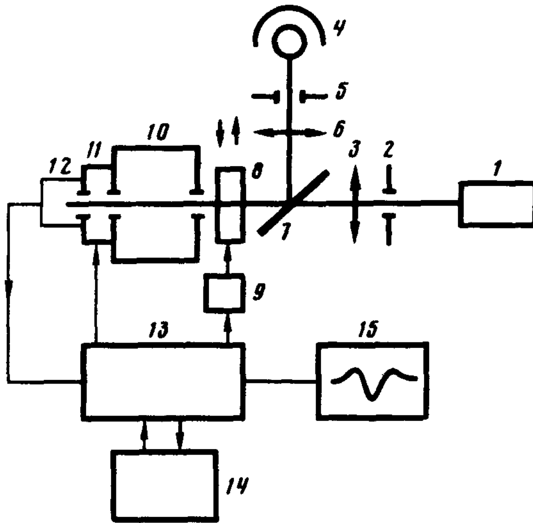

FIG. 2. Schematic of the experimental setup (top view): 1, LGI-21 nitrogen laser; 2 and 5, diaphragms; 3, lens and a periscope device for moving the laser focus in the vertical plane; 4, PRK-2 mercury tube; 6, quartz lens; 7, mirror (displayed measurement; the mirror is removed during irradiation); 8, cell; 9, a device for positioning the cell (the arrows indicate the direction of motion of the cell during the measurement); 10, SPM-2 monochromator (Karl Zeiss, Jena); 11, drive mechanism for the shutter, which shifts the photorecorder from the measuring beam to the reference beam; 12, FEU-39 photomultiplier; 13, KAMAK stand; 14, M-400 computer; 15, plotter.

For parameters of the NLM[2] and $\tau_{ir} < 5$ min, we estimated $P_S \lesssim 0.5$; thus, the probability of two breaks per molecule is low. For the given DNA, $\tau_1 = a^2/4D_0 \approx 10\text{ min} > \tau_{ir}$. Therefore, the conditions for applicability of Eq. (2) were satisfied qualitatively. With the passage of time τ_{ir} the laser was turned off and the recording system was turned on (Fig. 2). The cell was illuminated by a collimated light from a mercury tube. The entrance slit (7-μm opening) of the monochromator, which was turned to maximum DNA absorption ($\lambda = 254$ nm), was located directly behind the back plane of the cell. This slit was parallel to the direction of motion of the beam for NLM. A part of the light, which was transmitted through this slit past the cell, formed the reference beam. The spatial dependence of the optical density of DNA was recorded by shifting the cell perpendicularly to the monochromator's slit, using a precision mechanical system with a resolution of <4 μm. The control of the experiment and the evaluation and readout of the results of the measurements were done automatically using the real time of the M-400 computer via the KAMAK electronic system.

The main qualitative peculiarities of the results of the measurement, shown in Fig. 3, are: a valley $\Delta\rho$ at the center and peaks at the edges; an increase of $\Delta\rho$ at $t \lesssim t_1$ and a slow decrease at $t > \tau_1$; a spread of the valley and a faster spread of the peaks $\Delta\rho$ are in agreement with the theory (Fig. 1), just as are the values of τ_1 and P_S, which were calculated from the data in Fig. 3. An increase of $\Delta\rho/\rho$ by ≈ 0.02 in the irradiated volume is at variance with the theory (Fig. 3a). This increase, consistent with the hyperchromism of DNA produced due to NLM,[2] can be easily taken into account.

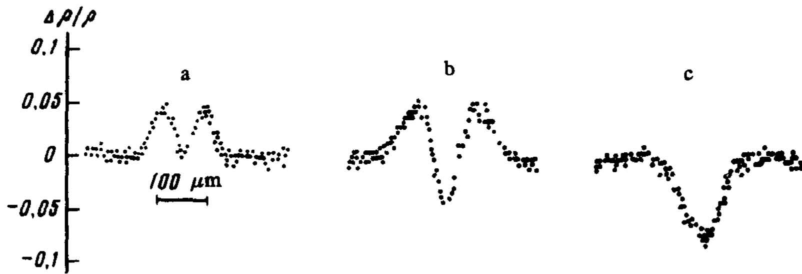

FIG. 3. Experimental spatial dependences of the optical density of DNA. The irradiation time is 5 min. Curves a, b, and c correspond to the times 0, 13, and 55 min after the irradiation. The curves were arranged sequentially for convenience. The points were plotted at 4-μm intervals.

The estimates show that the contribution from strictive forces and from thermal diffusion is negligible for our conditions. To eliminate these and other general effects, we performed a background experiment in which the 8-methoxi-6,7 furocoumarin dye with a similar structure and spectra, but which, judging by the differential spectrophotometry, does not form complexes with DNA. We managed to eliminate the effect in this manner.

The aggregate of the experimental data shows that the observed LID/DNA effect is produced according to the mechanism predicted theoretically. This effect can be used to investigate the interaction of strong radiation with DNA.

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[1)]The alternative possibility, which could not be excluded until now, is the formation of unstable bonds in the DNA molecules, which break subsequently of their own accord or under in the influence of external conditions. A gel electrophoresis, for example, which was used by Parkhomenko *et al.*[2] to observe the breaks, can produce such effect.

[2)]Since the optical density of DNA varies (increases) only several percents as a result of NLM,[2] we can assume with good certainty that it is proportional to the mass density of DNA.

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