Nonlinear laser cutting of RNA which is selective in terms of the set of bases

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A chromophore (dansyl) has been chemically attached to a small DNA fragment (an oligonucleotide) $(pT)_9$. In the presence of this compound, synthetic RNA molecules can be cut by the beam from a nitrogen laser, and the cutting can be carried out selectively in terms of the set of bases of the RNA. The effect is attributed to a radiationless transfer to the RNA of the energy of a two-photon excitation of the bound dye.

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The selective photomodification of macromolecules is of major interest in the physics of macromolecules, biophysics, biochemistry, and molecular biology. There is particular interest in the photomodification (including the rupture of bonds) of nucleic acids in regions with a given sequence of bases. It is not a simple matter to achieve selectivity in terms of position in a macromolecule because the monomer groups of any type are repeated many times in different parts of the macromolecule.

A complementary-addressing approach to modification has been developed for singling out a particular part of a nucleic acid for a selective effect.[1,2] The reaction group is chemically attached to the oligonucleotide address with a sequence of bases which is the complement of the sequence of interest. A Watson-Crick complex is formed by this oligonucleotide with the specified part of the nucleic acid, which is modified by the reaction group.

In the present experiments dye molecule was chemically attached to an oligonucleotide to arrange selective photomodification.[3] For illumination we used the beam from a near-IR laser, which is absorbed by the dye in a quasiresonant manner, but which is not absorbed by the nucleic acid. The energy of the stepwise two-photon excitation of the dye is transferred in a radiationless manner to the nucleic acid within a radius of 5 A (Ref. 4). The amount of energy trnsferred (6–8 eV) is sufficient to cause effective photomodification (in particular, to rupture a bond) of the nucleic acid. In this approach,[3–6] called "two-photon affine modification," a specificity of the binding in the macromolecule in terms of the chemical nature (affinity)[1,2] is combined with a high optical selectivity and the transfer of a large amount of energy upon a quasiresonant nonlinear excitation.[3,4]

The basic physics of the method of two-photon affine modification has been confirmed in model experiments with an unaddressed dye, which spontaneously aligns into a DNA double helix (in a random fashion along the length of the helix). It has

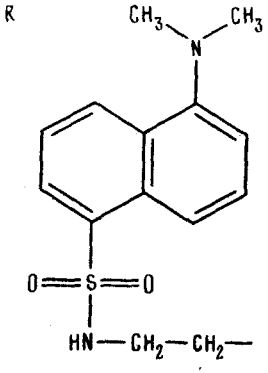

FIG. 1.

been shown that there is a nonlinear laser cutting of the DNA (a fragmentation of the DNA molecules).[5–7]

In the present experiments we have observed the first nonlinear laser cutting of RNA which is selective in terms of the set of bases and which involves an addressed dye. The dye has the structure $R(pT)_9$, where the radical R (Fig. 1), which includes a chromophore (dansyl), is bound to a phosphate at the 5′ end of an oligonucleotide $(pT)_9$, which contains nine thymine groups and which is therefore addressed in the sequence AAAAA...A in the nucleic acid. As the nucleic acids for these experiments we used synthetic homogeneous (i.e., containing bases of only one type) RNAs whose address is complementary and forms complexes with only the poly(A); as controls we used poly(C) and poly(U).

To study the nonlinear laser cutting we used the standard biochemical procedure of gel filtration and photoinduced diffusion of the nucleic acids.[8,9] This diffusion effect can be described as follows: The fragments of the nucleic-acid molecules which are formed during the nonlinear laser cutting diffuse out of the illuminated zone more rapidly than unbroken molecules can enter the illuminated zone from adjacent regions. The result is a spatial modulation $\Delta\epsilon(x)$ of the optical density of the nucleic acids, with a minimum in the illuminated zone and rises in the adjacent regions. The change $\Delta\epsilon$ is directly proportional to the number of cut molecules (at $|\Delta\epsilon| \ll 1$).

The experiments were carried out at 19 °C. The buffer solution was 10 mM tris·HCl, pH = 7.5; 10 mM $MgCl_2$; 0.2 M NaCl. The experimental apparatus and procedure with respect to the photoinduced diffusion were basically the same as in Refs. 8 and 9. The dimensions of the volume of solution illuminated by the LGI-21 laser were $\Delta y = 1$ mm along the beam direction, $\Delta z = 0.8$ cm in the vertical direction, and $\Delta x = 80$ μm in the transverse direction. The average beam power at the cell was 1.4 mW, and the pulsed power density was 70 MW/cm^2. The experimental results are shown in Fig. 2. The photoinduced diffusion is clearly expressed in the case of poly(A): $|\Delta\epsilon| \sim 0.1$. The spatial integral $\int \Delta\epsilon(x)dx$ is quite accurately zero, showing that the reason for the change in the optical density is the nonlinear laser cutting, not a degradation of the chromophores. There was absolutely no photoinduced diffusion in the control samples [poly(C) and poly(U)]. The small-scale fluctuations which can be seen, $|\Delta\epsilon| \lesssim 10^{-3}$, and which existed before the illumination, are attributed to inhomogene-

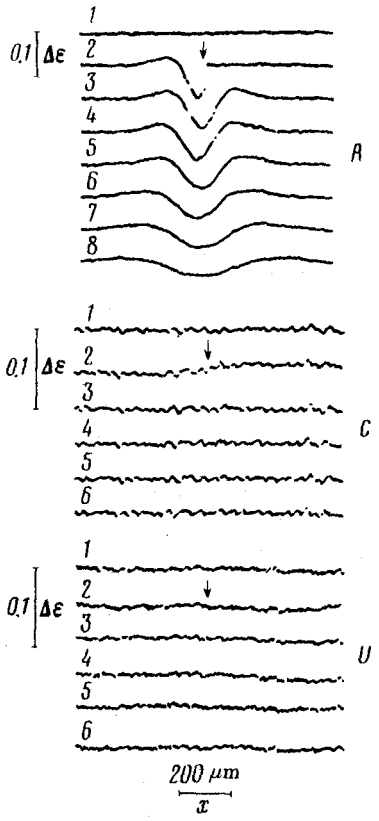

FIG. 2. Profiles of the optical density $\Delta\epsilon(x)$, $\lambda = 254$ nm. Here A, C, and U correspond to three experiments with poly(A), poly(C), and poly(U), respectively. 1—Before laser illumination; 2—laser illumination (3 min) at the point shown by the arrow; 3,4—recorded at 2-min intervals. The average optical densities of the cell are $\epsilon_{254} = 0.6$, $\epsilon_{337} = 0.09$. Equimolar mixtures of RNA with $R(pT)_9$.

ities of the cells. A photoinduced diffusion at the level of $|\Delta\epsilon| \gtrsim 10^{-3}$ would have been noticeable, since the selectivity of the photoinduced diffusion (and thus that of the nonlinear cutting) in terms of the set of bases is at least two orders of magnitude.

Further confirmation that the cutting is induced by the dye bound to the RNA comes from the agreement between the measured dependence of the magnitude of the effect ($\Delta\epsilon$) on the dye concentration and the theoretical prediction. The specificity of the cutting is also demonstrated by a concurrent inhibition: The effect was suppressed in an equilibrium mixture of poly(A) + $R(pT)_9$ + $(pT)_9$ at fixed concentrations of the first components with an excess of the third. We found that the magnitude of the effect is a quadratic function of the illumination intensity, in agreement with the unsaturated stepwise excitation.

The RNA samples illuminated at a power density of 150 MW/cm^2 (the beam source was an LGI-21 laser) were analyzed by gel filtration. With increasing illumination dose, the size distribution of the poly(A) molecules shifts markedly toward smaller

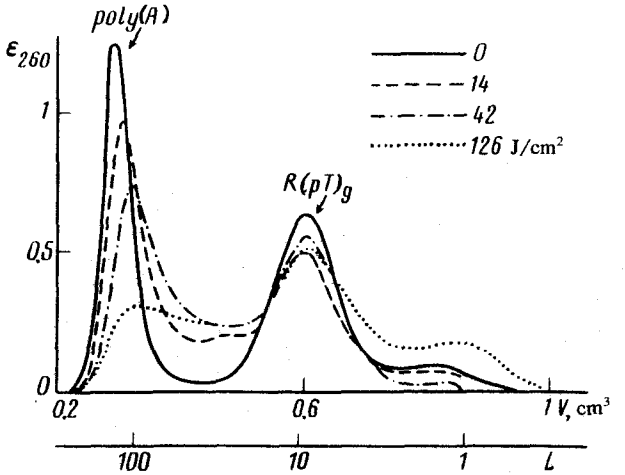

FIG. 3. Gel filtration of poly(A) + $R(pT)_9$ (in an equimolar mixture). The optical density ϵ_{260} as a function of the eluant volume V (0.3×25 cm column; Sephadex G-100). The estimated lengths L of the molecules are expressed in terms of the number of bases; the illumination doses received by the various samples are expressed in units of joules per square centimeter.

lengths (Fig. 3), and the initial and long molecules disappear. This experiment yielded an estimate of the RNA cutting rate in agreement with the data from the photoinduced diffusion. No important changes in the RNA distribution occurred in the control samples [poly(C) + $R(pT)_9$, poly(U) + $R(pT)_9$, and poly(A) without $R(pT)_9$].

In summary, using an addressed dye we have observed a nonlinear cutting of RNA; the selectivity of this cutting in terms of the set of bases is a consequence of the specificity of the Watson-Crick complementary complexes. The nonlinear laser cutting is induced by a variety of dyes (cf. Refs. 5–7), indicating that the transfer of excitation from the dye to the nucleic acid[4] is a universal basis for the phenomenon.

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