Two-quantum selective laser scission of polyadenilic acid in the complementary complex with a dansyl derivative of oligothymidilate

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Nonathymidilate was synthesized containing the chromophore (dansyl) group linked to its 5'-phosphate. In the presence of this compound the polyadenilic acid molecules are split by the radiation (power density $J \geq 70$ MW/cm^2) of a nitrogen laser, while under the same conditions poly(C) and poly(U) are hardly affected. This selective optically non-linear effect was predicted and is explained in terms of radiativeless transfer of two-quantum excitation of the chromophore which is fixed on poly(A) molecule due to the formation of the complementary complex with nonathymidilate.

Laser scission *Polyadenilic acid* *Complementary complex*
Two-quantum excitation of the chromophore *Dansyl derivative of nonathymidilate*
Light-induced diffusion

1. INTRODUCTION

Selective modification of biopolymers is of great interest for molecular biology, biochemistry, biophysics and the physics of macromolecules. Photomodification possesses obvious merits of simplicity, high efficiency, possibility to localize excitation in time to picoseconds and in space to a micron size. The difficulty of providing the selectivity, i.e., to photomodify the macromolecule in a definite site, is mainly due to multiple repetition of the monomer residues of one type in different regions of the macromolecule.

To affect a required region of a nucleic acid (NA) molecule the method of complementary addressed modification had been developed [1,2]. This consists in the following: a reactive group is chemically linked to an address; i.e., the oligonucleotide with the base sequence which is complementary to the required one. The modification of NA (chemical interaction of the bound reactive group with NA) occurs specifically near the position of the complementary complex between the oligonucleotide and NA; i.e., in the required region.

To provide the selective photomodification, a chromophore (dye) group, instead of the reactive one, should be chemically attached to the oligonucleotide address [3,4]. Irradiation is carried out with soft UV ($\lambda > 300$ nm) laser light which is not absorbed by NA, but is quasi-resonantly absorbed by the dye. If the radiation intensity J is moderately high ($J \geq 50$ MW/cm^2), the stepwise two-quantum excitation of the dye occurs with an

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Abbreviations: NA, nucleic acid; LID, light-induced diffusion; UV, ultraviolet; TAM, two-quantum affinity modification; DnsAE or dansylaminoethyl, 2-*N*-(5-*N*-dimethyl-1-naphthalenesulfonyl)aminoethyl

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appreciable probability, and then the excitation energy is radiativelessly transferred to NA in a vicinity of ≤ 5 Å around the dye group [4]. This energy (6–8 eV) is sufficient to efficiently modify NA (in particular to break its chain). Thus, this approach [4], called the two-quantum affinity modification (TAM) method, unifies high chemical specificity of the affinity binding to the macromolecule [1,2] with high optical selectivity and large energy transfers which are characteristic of the quasi-resonant non-linear excitation [3,4].

The physical principles of TAM had been confirmed in model experiments [5–7] using the dye 8-methoxipsoralen which was known to intercalate the DNA duplex (in random sites) [8]. The phenomenon of non-linear laser scission of DNA, i.e., laser-induced fragmentation of DNA molecules in the optically non-linear process, had been established [5,6]; this proved to occur via the bound dye only [7].

Here, for the first time the non-linear laser scission of polynucleotide (poly(A)) is observed with the use of the addressed dye DnsAE(pT)$_9$; i.e., the nonathymidilate derivative containing the dansyl group linked to its 5′-phosphate. The scission was practically absent for poly(C) + DnsAE(pT)$_9$, poly(U) + DnsAE(pT)$_9$, and poly(A) in the absence of DnsAE(pT)$_9$, which served as the control.

2. MATERIALS AND METHODS

To study the scission of polynucleotides gel chromatography and a new approach based on the recently established effect of the light-induced diffusion (LID) of NA [9–11] was employed. The latter consists in the following:

Let a limited region in the NA solution be laser-irradiated to induce the scission of NA molecules. Then shorter (cut) molecules of NA appear which possess greater diffusion coefficients and diffuse out from this region faster than intact (large) molecules penetrate into it from outside. As a result, the mass density and the absorbance ϵ of NA decreases in the irradiated volume and increases in adjacent regions. This very effect has been called LID NA [9,10].

The theory of LID (to be published) shows that the variation $\Delta\epsilon$ of A (for $|\Delta\epsilon| \ll 1$) is proportional to the total number of the cut NA molecules. A merit of LID is its applicability to detect scission in polydisperse NA solutions, where molecules of different sizes are present, as distinct from gel chromatography.

Polynucleotides were from Reanal. For the experiments with gel chromatographic analysis these were gel-chromatographed prior to irradiation to isolate monodisperse fractions with nearly equal sizes of the molecules; a Sephadex G-100 superfine (Pharmacia) column (0.3 × 25 cm) was used, 0.1 ml of the 1 mM polynucleotide solution and/or 1 mM of DnsAE(pT)$_9$ (the mononucleotide concentrations) in the buffer (10 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 0.2 M NaCl) was irradiated in a quartz cuvette (C, Zeiss, Jena) of 1 mm thickness (in the laser beam direction), the laser was LGI-21 (USSR), $\lambda = 337$ nm, radiation mean power, $W = 3$ mW, the pulse power density $J \approx 150$ MW/cm^2. After receiving certain doses (uniformly spread over the cuvette by scanning) 30 μl aliquots were isolated and analyzed by gel chromatography.

The set up and the course of the LID experiments were similar to those in [9–11]; $J \approx 70$ MW/cm^2, $W = 1.4$ mW; the cross-section of the irradiated region had dimensions: vertical $\Delta z = 0.8$ cm, horizontal $\Delta x = 80$ μm. After a short irradiation the laser was switched off, and the spatial distributions of the $A\,\Delta\epsilon(x)$ were recorded at intervals of a few min with an inaccuracy less than 0.1% and a spatial resolution of 10 microns.

UV spectra and the melting curves of the complementary complex were recorded with DU-8 (Beckman) and Gilford-250 spectrophotometers. The fluorescence was studied with a MPF-4 (Hitachi) spectrofluorimeter. The reversed-phase and gel chromatographies were carried out with a 'Millichrom' liquid chromatograph (Orel).

Nonathymidilate was synthesized by the triester method using a condensation between the 3′-OH group of the OH-component and 5′-*p*-chlorophenyl ester of the *p*-component in the presence of 2,4,6-triisopropylbenzenesulphonyl chloride and *N*-methylimidazole in absolute chloroform similar to [12]. The fluorescent group was linked to nonathymidilate with the reaction of condensation of 2-*N*-(5-*N*-dimethylamino-1-naphthalenesulphonyl)-aminoethanol and 5′-*p*-chlorophenyl phosphomonoester group of nonathymidilate under the same conditions. Details of the synthesis are described in [13].

3. RESULTS AND DISCUSSION

The absorption and fluorescence spectra of DnsAE(pT)$_9$ are shown in fig.1. From the melting A curve (fig.2) of an equimolar mixture of poly(A) with DnsAE(pT)$_9$ the dissociation constant of the complementary complexes at 18°C was extracted: $K_d = (1.3 \pm 0.5) \times 10^{-6}$ M.

The results of the experiments on LID of the polynucleotides are shown in fig.3. In the case of poly(A) the LID effect is strongly pronounced: the magnitude of the A spatial modulation $\Delta\epsilon_m = \epsilon_{max} - \epsilon_{min}$ (λ = 254 nm) reaches 0.1 (signal: noise ratio $\approx$ 100); the integral $\int\Delta\epsilon(x)dx$ with good accuracy is zero. The latter fact confirms that the A change is just caused by the laser-induced chain scission and not by chromophore degradation.

In contrast to the above, the LID effect for poly(C) and poly(U) is completely absent despite a 2-fold increase of the resolution in $\Delta\epsilon$. Some small-scale fluctuations $|\Delta\epsilon| \approx 10^{-3}$, which can be seen in fig.3 (C, U) are due to inhomogeneities of the cuvettes. A small bend of curve 2 (C) in the irradiation point is due to a random drift of the probing light (of the Hg lamp) during the irradiation; much shorter scanning time is not sufficient to manifest this drift in the other curves. Let us point out that LID with the magnitude $|\Delta\epsilon| \gtrsim 10^{-3}$ would already be detectable. Hence, the observed selectivity of LID and, consequently that of the laser scission, in the polynucleotide type is, at least, two orders of magnitude.

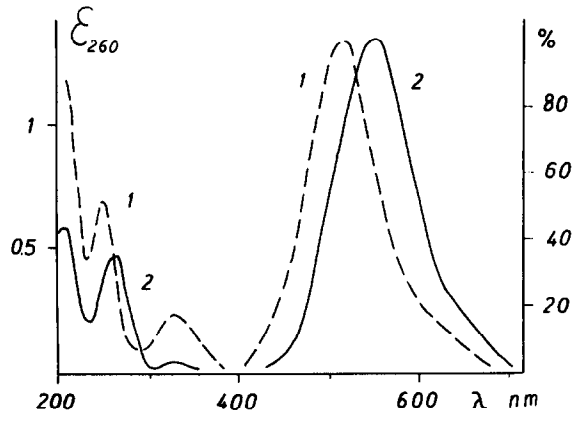

Fig.1. The absorption (to the left) and fluorescence (to the right, λ_{exc} = 337 nm) spectra of dansylaminoethanol in methanol (1) and DnsAE(pT)$_9$ in water, pH 6.0 (2).

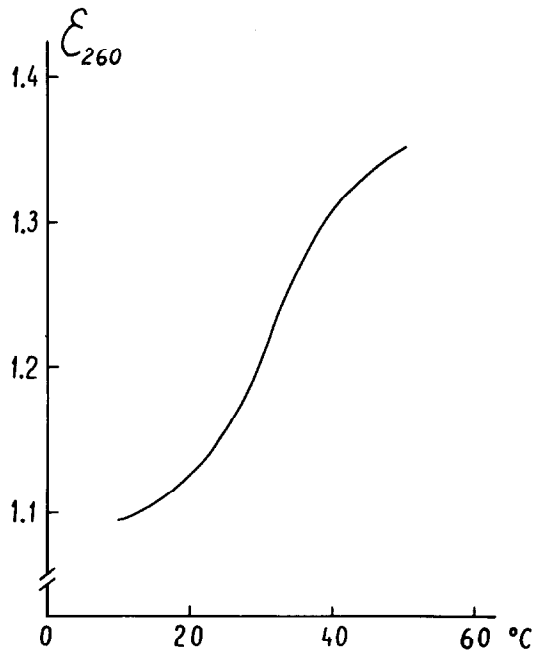

Fig.2. The melting profile (A against the temperature) of the equimolar mixture of poly(A) with DnsAE(pT)$_9$.

At the present temperature (18°C) most ($\approx$80%) the DnsAE(pT)$_9$ molecules are in the complementary complexes with poly(A) (cf. fig.2). This supports the suggestion that the laser scission of poly(A) is only induced by DnsAE(pT)$_9$ bound to poly(A). This suggestion is directly confirmed by the observed dependence of the LID effect on the concentration of DnsAE(pT)$_9$ (fig.4). The theoretical curve has been calculated on the basis of the above suggestion for the value $K_d = 1 \times 10^{-6}$ M, which is in agreement with the presented melting data.

The specificity of the laser scission of poly(A) is also proved by the observed competitive inhibition: in the equilibrium mixture poly(A) + DnsAE(pT)$_9$ + (pT)$_9$ at fixed concentrations of the first two components and at an excess of the third one, the LID effect was significantly suppressed.

The quadratic dependence of the magnitude of the LID effect on the power of the exciting radiation (fig.5) indicates the optically-non-linear (non-saturated two-quantum) mechanism of the scission excitation (cf. [4]).

The results of the experiments with gel chromatographic analysis (fig.6) are in perfect qualitative agreement with what was observed by

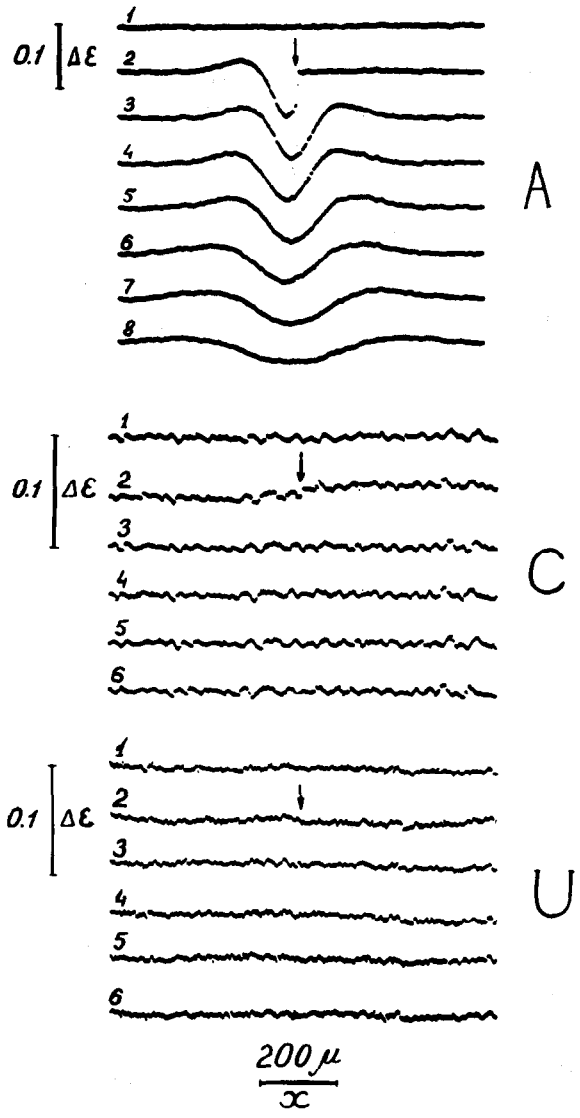

Fig.3. The results of the LID experiments; the spatial distributions of the variation of the solution A $\Delta\epsilon$ (λ = 254 nm) against the coordinate x recorded at different times; A, C, U correspond to the experiments with poly(A), poly(C) and poly(U), respectively. (1) Before the laser irradiation; (2) the A was scanned from the right to the left, then the scanning was interrupted and the laser irradiation was carried out for 3 min at the point indicated by the arrow, after that the scanning was continued; (3,4...) the A was recorded at 2-min intervals (the scanning time was 26 s/curve). The equimolar mixture of the polynucleotides with DnsAE(pT)$_9$, the mean A's of the cuvette: ϵ_{337} = 0.09; ϵ_{254} = 0.6.

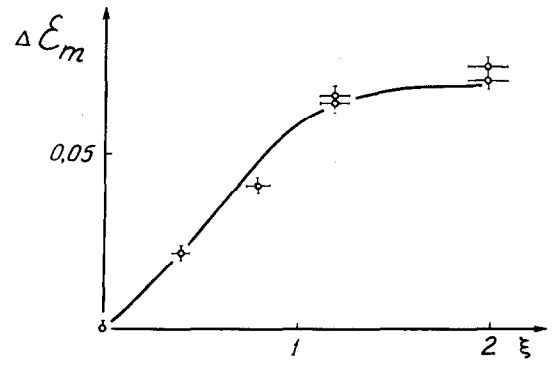

Fig.4. The magnitude of the LID effect $\Delta\epsilon_m$ as the function of relative concentration of DnsAE(pT)$_9\xi$ (ξ = 1 corresponds to the equimolar mixture). The concentration of poly(A) is 2×10^{-4} M. Circles denote experimental points and the bars the estimated errors.

the LID method. In the case of poly(A) with the dose increase one can see a gradual reduction of the intensity of the peak (1 in fig.6) of the original molecules concurrent with its broadening and shift toward the region of shorter molecules. This is just what is expected for the laser scission (in random sites of molecules, which is the case for the present experiments). Moreover, the scission efficiency, which can be estimated from these data, can be shown to be in quantitative agreement with the results of the LID experiments.

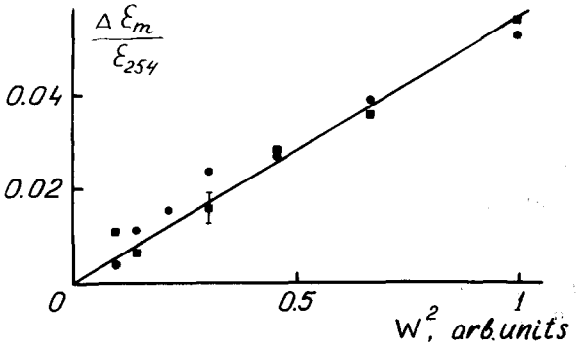

Fig.5. The relative magnitude of the LID effect $\Delta\epsilon_m/\epsilon_{254}$ as the function of the mean laser radiation power incident on the cuvette W_c. The maximum value W_c = 1.4 mW (the corresponding maximum pulse intensity J $\approx$ 70 MW/cm^2). The circles and quadrangles correspond to two independent experiments. The straight line is obtained by χ^2-fit. The typical statistical error is indicated by bars.

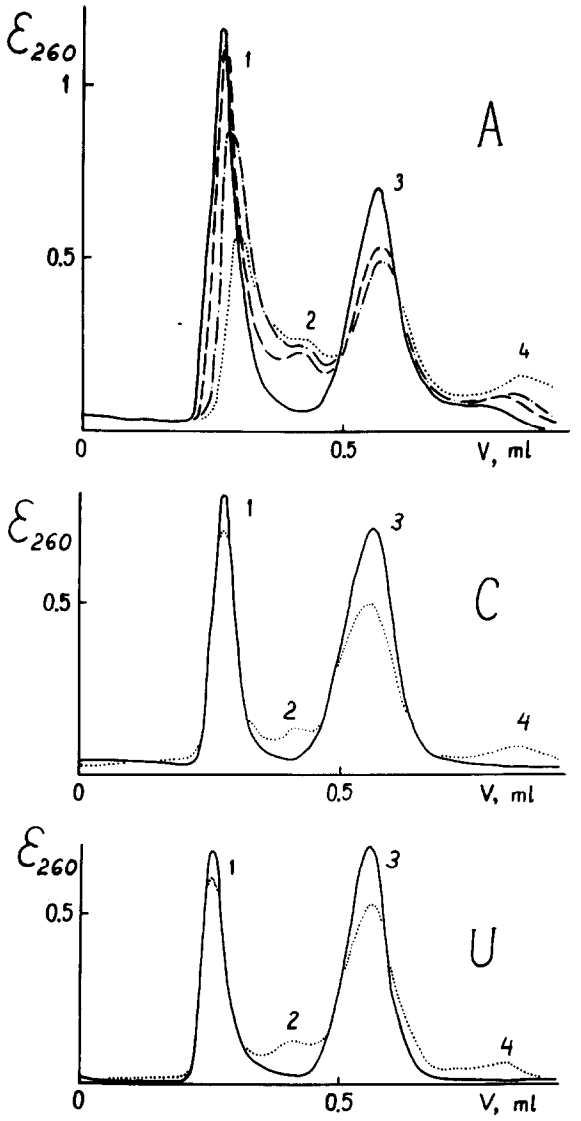

Fig.6. The gel chromatography on the Sephadex G-100 superfine: the solution *A* ϵ_{260} against the volume of the eluent *V*. A, C, U correspond to the independent experiments with poly(A), poly(C), poly(U), respectively, all in the equimolar mixture with DnsAE(pT)$_9$. (——), (– – –), (– – – –) and (···) correspond to irradiation doses of 0 (initial solution), 4.7, 14 and 42 J/cm^2, respectively. The column 0.25 × 17 cm, elution by 7 M urea at 0.59 ml/h.

In the cases of poly(C) and poly(U) (fig.6C,U) and also in the separate experiment with the irradiation of poly(A) in the absence of DnsAE(pT)$_9$, no appreciable changes of the polynucleotide peaks, either in their positions or in their width, were observed even for the maximum dose of 42 J/cm^2. Small peaks 2 (fig.6, A,U), which appear on the irradiation and are observed, irrespective of the type (or even of presence of polynucleotide as it was shown separately), are probably due to nonathymidilate dimerization. Peak 3 is attributed to DnsAE(pT)$_9$ and peak 4 to low-M_r products of the degradation of DnsAE(pT)$_9$ and, probably, of poly(A) as well. Thus no laser scission occurs unless there are conditions for complementary complex formation.

In such a way, for the first time we observed the laser scission of a polynucleotide chain in the optically non-linear process mediated by the dansyl derivative of an oligonucleotide. This was studied by two independent methods. The fact that the laser scission is mediated by the chromophore groups, which are chemically essentially different (cf. [5–7]), provides evidence for the universal mechanism [4] of this process. The scission is highly selective in the polynucleotide type: the non-specific (in the absence of the complementary complexes) scission is by two orders of magnitude, at least, less efficient than the specific one, if it occurs at all. The results obtained clearly indicate the possibility employing the developed approach for the complementary addressed laser scission of nucleic acids, in particular to study their structure and function.

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