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MODIFICATION OF DNA BY PHOTOACTIVABLE ARYL AZIDES

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Features of the interaction with DNA of photoactivable aryl azides having various substituents in the aromatic ring have been studied. The nature of the interaction with DNA and the degree of its modification depend substantially on the nature of these substituents. The results obtained can be used for obtaining DNA probes bearing various marker groups.

The study and use of natural biological compounds is connected to a considerable degree with their capacity for being modified under the action of various chemical agents. In this respect, the interaction of photoactivable reagents such as aryl azides with nucleic acids has been studied comparatively little. These compounds, on irradiation with light of various wavelengths, generate highly reactive nitrenes [1, 2], which interact with nucleic acids [3, 4]. In the present paper, we report features of the interaction with DNA of photoactivable aryl azides having various substituents in the aromatic ring. It has been shown that the nature of the interaction with DNA and the degree of its modification by the aryl azides depend substantially on the nature of these substituents. The results obtained can be used to obtain DNA probes bearing various marker groups.

As photoactivable reagents we synthesized the compounds the structural formulas of which are shown in Fig. 1. The choice of compound was determined by the aims of the present investigation: to study the influence of a) the presence of a nitro group in the aromatic ring, and b) the nature of the radical R' on photolysis and the interaction of the products of the photolysis of the azides with single- and double-stranded forms of DNA on irradiation with visible light.

In the first stage of the work we studied the influence of substituents in the aryl azide and the composition of the buffer on the characteristics of photolysis. Below, we give the half-decomposition times of the azide (I) determined from the curve of the dependence of the decrease in the absorption of a solution at 268 nm on the time of irradiation using a number of light-filters:

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Filter range of transmission, nm	$T^{1/2}$, min
without a filter	0,10±0,05
UFS6 (300—400)	0,3 ±0,1
SS8 (340—520)	3,0 ±0,5
S3SF (340—800)	2,5 ±0,5
3S8 (400—700)	8,5 ±0,5
5S-8 (>380)	10,0 ±0,5

Azide (I) was decomposed extremely effectively by visible light. Similar results were obtained in a study of the photolysis of azides (II) and (III). The half-decomposition times of azides (I)-(III), each containing a nitro group, did not depend on the nature of the radical R'. The use of any filters restricting the passage of UV radiation, even partially, led to a substantial decrease in the rate of photolysis of the azides (IV) and (V). At $\lambda \geq 400$ nm an appreciable effect was observed only after irradiation for several hours. Since the irradiation of DNA with UV light leads to considerable changes in its structure, analysis of the modification of DNA was carried out with the use only of azides (I)-(III), using a filter with a transmission interval of 340-800 nm. For azide (I) we studied the dependence of the time of half-decomposition on the pH and the composition of the buffer. The rate of photolysis of azides (I) did not depend on the pH of the medium (in the interval from 2 to 12 pH units) nor on the ionic strength in the range of 0-0.2 M NaCl, nor on whether a sodium acetate or a sodium phosphate buffer was used. We subsequently used a 0.1 M sodium phosphate buffer, pH 7.4.

To study the interaction of the azide with DNA, different amounts of the reagents were mixed with 1-3 μg of plasmid DNA and the mixtures were subjected to photolysis with visible light. The ratios of the molar concentrations of the azide and the DNA (calculated to a base), r, and the time of the irradiation, τ, were varied. For a rough estimate of the degree of modification of the DNA by photoreagents (I) and (III), biotin was added to the latter after the performance of the photolysis reaction (see the Experimental part), and this was then revealed colorimetrically [5, 6]; photoreagent (II) contained a biotin label initially. The degrees of modification of the DNA by reagent (I) and by reagents (II) and (III), differed substantially, as follows from comparison of the intensities of the signals obtained when the samples of DNA containing biotin were treated with the avidin—alkaline phosphatase system on nitrocellulose filters (Fig. 2). Thus, in spite of the fact that the value of r in the case of azide (II) was 20 times that for azide (I), at equal values of τ, the degree of modification of the DNA by azide (I) was approximately an order of magnitude greater than by azide (II) (Fig. 2). When azides (I)-(III) interacted with the single-stranded form of DNA, regardless of the nature of R', a substantial increase in the degree of modification of the DNA was observed (Fig. 2). However, in this case, as well, azide (I) modified the DNA to a far greater degree than azides (II) and (III). Quantitative results on the interaction of reagent (I) with DNA were obtained with the aid of the reaction with fluorescein isothiocyanate with the DNA that had been labeled with azide (I) [7]. At r = 0.5 and τ = 2-3 min, on photolysis one molecule of the azide (I) interacted with each 120 ± 20 pairs of bases in the DNA.

The samples of DNA obtained on interaction with the azides under various conditions were characterized with the aid of electrophoresis in agarose gel. When reagent (I) was used, analysis of the samples of DNA obtained was carried out after their biotinylation

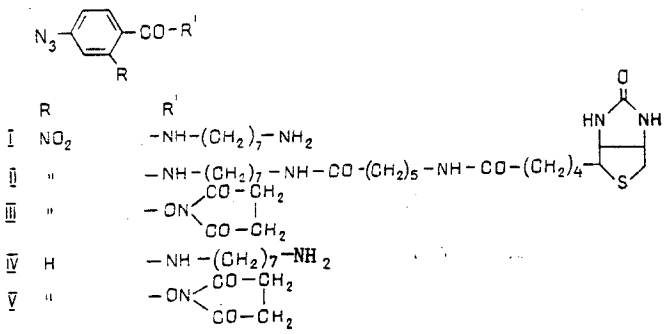

Fig. 1. Structural formulas of the azides under consideration.

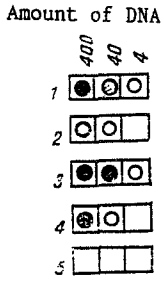

Fig. 2

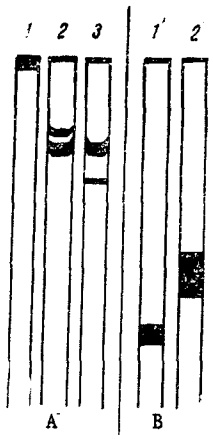

Fig. 3

Fig. 2. Determination of biotinylated DNA on nitrocellulose filters using avidin and alkaline phosphatase: 1) double-stranded DNA modified with the aid of azide (I) (τ = 2-3 min, r = 0.5); 2) double-stranded DNA modified with the aid of azide (II) (τ = 2-3 min, r = 10 min); 3) single-stranded DNA modified with the use of azide (I) (τ = 2-3 min, r = 0.5); 4) single-stranded DNA labeled with the aid of azide (III) (τ = 5 min, r = 10); 5) unlabeled DNA. The amounts of DNA deposited are shown in the figure.

Fig. 3. Electrophoretic analysis of biotinylated DNA in agarose gel: A and B) single- and double-stranded forms of DNA, respectively; 1, 2, and 1', 2', respectively) samples of DNA obtained under various conditions of biotinylation (see text); 3) unmodified DNA.

in order to exclude the influence of the positive charge on the mobility of the DNA. The addition of a biotinylated label to double-stranded DNA modified with azide (I) led to a considerable decrease in the mobility of the DNA in 0.4% gel, the effect being more pronounced the greater the values of r and τ (Fig. 3A, 1 and 2). The bulk of the DNA obtained at r = 1.0 and τ = 5 min did not pass into the gel (Fig. 3A, 1). However, the disappearance of the band corresponding to the superspiralized form indicates the introduction of breaks into the DNA even at r = 0.08 and τ = 15 sec. The difference in the degrees of fragmentation arising in the photo-induced reaction under various conditions can be estimated qualitatively with the aid of electrophoretic analysis of single-stranded forms of the DNA. In spite of the high degree of loading of the DNA with biotin under "severe" reaction conditions, the mobility of its single-stranded form was substantially higher than that of DNA having a smaller load (Figs. 1, 2, and 3), i.e., the DNA obtained at r > 1 and τ > 5 min contained considerable amounts of biotin but at the same time was fragmented to a far greater extent than the DNA biotinylated under milder conditions. The electrophoretic properties of the plasmid DNA scarcely changed after its modification by azides (II) and (III) taken in concentration 20 times exceeding the concentration of azide (I).

The facts presented above, taken all together, lead to the conclusion that although the photolysis of the azides studied was not affected by the nature of the radical R', their capacities for modifying DNA are different. The presence of the R' of a positive charge substantially changed the nature of the interaction of the products of the photolysis of the azides with DNA. There is no doubt that a positive charge in the azide molecule leads to an increase in the local concentration of the reagent close to the DNA and, consequently, to an increase in the degree of its modification. This effect appeared on the interaction of an aryl azide both with double-helical and with single-stranded forms of DNA. At the same time, the high degree of modification of single-stranded DNA observed regardless of the nature of the radicals studied permits the assumption that in the case of double-helical DNA the products of the photolysis of the aryl azides did not interact with groups participating in the formation of hydrogen bonds.

The occurrence of the fragmentation of DNA in the presence of small amounts of the azide (I) and its absence on the photolysis of azides (II) and (III) taken in large excess cannot, in our opinion, be explained only by the increase in the concentration of azide

(I) close to the DNA. The positive charge can apparently bring about a definite orientation of the azide (I) molecules relative to the phosphodiester bond in the DNA, which leads to a cleavage of this bond in the photolysis of the aryl azide. It is also possible that azide (I), interacting with definite groups of the DNA, causes a decrease in the strengths of the glycosidic bond in the modified DNA, as a result of which its apurinization and, as a consequence of this, the fragmentation of the DNA take place.

EXPERIMENTAL

We used 4-aminobenzoic acid and avidin from Serva, 1,7-diaminoheptane and dicyclohexylcarbodiimide (DCC) from Ferak, Silufol plates, alkaline phosphatase, Nitrotetrazolium Blue (NBT), and 5-bromo-4-chloroindol-3-yl phosphate (BCIP) from Boehringer, and Schleicher and Schüll nitrocellulose filters. DNA was isolated from the plasmid pCR-I by a standard procedure [8].

Synthesis of N-(4-Azido-2-nitrobenzoyl)-1,7-diaminoheptane (azide I). The 4-amino-2-nitrobenzoic acid was obtained from 4-aminobenzoic acid [9]. The N-hydroxysuccinimide ester of 4-azido-2-nitrobenzoic (azide III) was obtained by the general procedure described in [10]. The presence of the azide group was confirmed by the appearance in the IR spectrum of a characteristic azide band at 2140 cm^{-1} with a small shoulder at 2120 cm^{-1}. UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 285, 335 nm. A solution of 1 mmole of the N-hydroxysuccinimide ester of 4-azido-2-nitrobenzoic acid in 3 ml of dioxane was added dropwise to 2 mmole of 1,7-diaminoheptane in 3 ml of dioxane, and the mixture was stirred vigorously at 20°C. The resulting precipitate was separated off by centrifugation and was washed twice with dioxane, and all the fractions of the supernatant were combined. The dioxane solution was diluted fivefold with 0.1 M hydrochloric acid, and the small precipitate that formed was removed by centrifugation. Additional purification was carried out with the aid of chromatography on Silufol plates in the chloroform–ethanol (8:2 by volume) system. A UV-absorbing zone with a different mobility from those of benzoic acid and its N-hydroxysuccinimide ester was seen on the chromatogram. The presence of a primary amino group was confirmed by the ninhydrin reaction. UV spectrum: $\lambda_{max}^{H_2O}$ 260, 370 nm. All the operations with the azide were carried out in a darkened room. The reagent obtained could be stored for not less than eight weeks at −5°C.

N-(4-Azidobenzoyl)-1,7-diaminoheptane (azide IV) and the N-hydroxysuccinimide ester of 4-azidobenzoic acid (azide V) were obtained by a similar procedure. Azide (II) was obtained by the reaction of equimolar amounts of azide (I) with the N-hydroxysuccinimide ester of ε-biotinylaminocaproic acid in dimethylformamide. The purity of the product obtained was checked by chromatography on a Silufol plate in the chloroform–ethanol (8:2) system.

Modification of DNA with the Aid of the Azides (I)-(V). In a quartz cell with a diameter of 0.5 cm, 400 µl of a solution of DNA in 0.1 M phosphate buffer, pH 7.4, having a concentration of 0.2-2.0 mM was mixed with 2-20 µl of a 0.03 M solution of an azide in DMFA, and the mixture was irradiated with a KhVO-150 lamp from a distance of 4 cm using various filters. The cell was cooled with ice. Then 30 µl of 1 M phosphate buffer, pH 9.0, was added and the mixture was extracted twice with sec-butanol. After this, the DNA was precipitated with 70% ethanol in the presence of 0.25 M sodium acetate.

Biotinylation of DNA with the Aid of Reagent (I). The photolysis of reagent (I) in the presence of DNA was carried out as described above. The DNA was dissolved in 0.1 M phosphate buffer, pH 8.5, and a tenfold excess (with respect to the bases of the DNA) of the N-hydroxysuccinimide ester of ε-biotinylaminocaproic acid was added. The mixture was kept at 20°C for 1 h and was then extracted twice with sec-butanol and the DNA was precipitated with 70% ethanol.

Biotinylation of DNA with the Aid of Azide (III). The photolysis of the reagent at 0°C in the presence of DNA was carried out as described above. Then, immediately, a tenfold [with respect to the amount of azide (III)] excess of 1,7-diaminoheptane was added and the mixture was kept at 20°C for 1 h. It was then extracted twice with sec-butanol at pH 9.0 and the DNA was precipitated with ethanol, after which it was dissolved in 0.1 M phosphate buffer and was treated with the N-hydroxysuccinimide ester of ε-biotinylaminocaproic acid.

Preparation of Fluorescein-Labeled DNA. The method of [7] was used with slight variations. A precipitate of DNA modified with azide (I) was dissolved in 0.1 M phosphate buffer,

pH 7.2. A freshly prepared solution of fluorescein isothiocyanate in dimethyl sulfoxide (10 mg/ml) was added to a solution of the DNA (I) in 20-fold excess. The mixture was incubated in the dark at 37°C for 3 h. The complete washing out of the reagent that had not become bound to the DNA required eight precipitations with 80% ethanol. The completeness of the elimination was checked spectrofluorometrically. The degree of modification of the DNA by the fluorescein was calculated as described in [7].

Immobilization of DNA on Nitrocellulose Filters. A filter was washed twenty times with buffer A (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and dried, a solution of DNA in 0.1 mM EDTA was added and it was again dried and was baked at 80°C for 3 h.

Analysis of Biotinylated DNA on Filters. A published method [11] was used with the difference that the filters were treated with avidin, after which they were washed additionally with buffer A (3 × 5 min). After the filters had been treated with biotinylated alkaline phosphatase, they were additionally washed with a buffer containing 3 M NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 0.03 M triethanolamine, pH 7.7.

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SYNTHESIS OF THE ACETATE OF OCTADECA-trans-2,cis-13-DIEN-1-OL, A COMPONENT OF THE SEX PHEROMONES OF Synanthedon tipuliformis AND Zeuzera pyrina

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A new synthesis of the acetate of octadeca-trans-2,cis-13-dien-1-ol based on the alkylation of propargyl alcohol with pentadec-cis-10-enyl bromide, obtained from the readily accessible decamethylene bromohydrin, has been effected.

The sex pheromone of the current clearwing moth Synanthedon tipuliformis, one of the main pests of redcurrants and blackcurrants has been identified as a mixture of the acetates of octadeca-trans-2,cis-13-dien-1-ol (VII) and of octadec-cis-13-en-1-ol (VIII) in a ratio of 93:7 [1, 2]. A mixture of the two acetates is attractive for males of the pests under field conditions, and so also is the dienic acetate (VII) alone.

The sex pheromone of the leopard moth Z. pyrina, a dangerous trunk pest of apple and pear trees and other fruit crops, has been identified as a mixture of the dienic and

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