THE EFFECT OF HIGH INTENSITY ULTRAVIOLET IRRADIATION ON NUCLEIC ACIDS AND THEIR COMPONENTS

Cleavage of *N*-glycosidic bond in thymidine, adenosine and 2′-deoxyadenosine

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1. Introduction

The biological consequences of ultraviolet irradiation of cells and viruses were conditioned mainly by photochemical alterations of the nucleic acid components [1]. The quantum yield of cell inactivation was recently shown to increase with increasing intensity of the irradiation from 10^{23}–10^{25} photons . cm^{-2} . s^{-1} (λ = 266 nm) [2]. Irradiation at such intensities results in noticeable photoconversion of thymine and adenine by 2-photon processes [3,4]. These data allow one to suggest that the change of quantum yield of cell inactivation upon high intensity ultraviolet irradiation is conditioned by the 2-photon conversions of cellular DNA components. The close similarity of products formed in the aforementioned conditions and upon γ-irradiation of dilute aqueous thymine solutions [3] indicates a similar character of at least some DNA lesions in both these cases. It should be emphasized that due to different spectral characteristics of nucleic acids, proteins, polysaccharides and other components of cells and viruses, the main targets of high intensity ultraviolet irradiation are nitrogen bases of polynucleotides. At the same time ionizing radiation usually induces simultaneous damages of all the intracellular components, including bases and sugar moieties of polynucleotides as a result of both direct radiolysis and reactions mainly with the products of radiolysis of water and other cell components [5].

In polynucleotides the 2-photon processes induced by high intensity ultraviolet irradiation may plan an important role resulting not only in degradation of nucleic acid bases but in some other conversions. These data provide evidence that high intensity ultraviolet irradiation of aqueous solutions of thymidine, adenosine and 2′-deoxyadenosine brings about simultaneous degradation of nucleic acid bases and cleavage of *N*-glycosidic bonds.

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2. Materials and methods

The radiochromatographic purity of [2-^{14}C]thymidine (Isotope, Leningrad, 50 mCi/mmol) and [U-^{14}C]adenosine and 2′-deoxy-[U-^{14}C]adenosine (Czechoslovakia, 445 and 77 mCi/mmol, respectively) after purification by preparative thin-layer chromatography (TLC) in solvent systems (see below) A and B for thymidine and C for adenine nucleosides exceeded 98%.

Nucleosides dissolved in bidistilled water ($\sim 10^{-4}$ M, pH 6.5) were irradiated (pathlength 0.2 cm) with vigorous stirring at room temperature. A 4th harmonic of a YAG laser was used as a 266 nm ultraviolet source (1.3×10^{24} photon . s^{-1}, pulse duration 10 ns, pulse repetition frequency 1 s^{-1}) [2,3].

Nucleoside solutions were analysed by TLC (silica gel plates 'Silufor UV-254', Czechoslovakia) in the following solvent systems: A, ethyl acetate–isopropanol–water (75 : 16 : 9); B, chloroform–methanol–water (4 : 2 : 1, lower layer with 5% methanol added); C, water; D, *n*-butanol–water (86 : 14); E, *n*-butanol–water–acetic acid (5 : 3 : 2); F, isopropanol–concentrated ammonia–water

(7 : 2 : 1). Chromatograms were cut into 0.5 × 1.0 cm rectangles and radioactivity of each rectangle measured with an SL-30 scintillation counter (Intertechnique, France). Compounds were identified by their mobilities in 3 different solvent systems. The identity of ultraviolet-absorbing products was additionally confirmed spectrophotometrically after elution of the respective spots with water. Sugars on chromatograms were detected by the anisaldehyde reagent [7].

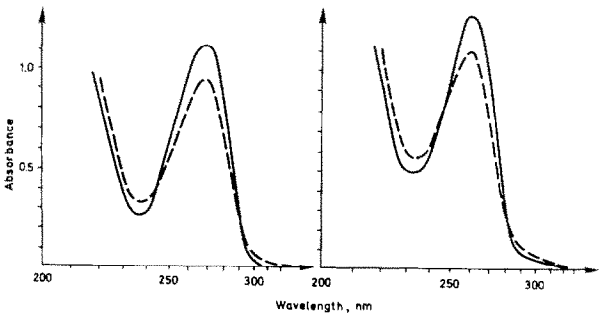

Fig.1. Ultraviolet absorption spectra of dilute aqueous solutions of thymidine (left) and adenosine (right) before (full lines) and after (dotted lines) irradiation by high intensity ultraviolet light (λ = 266 nm, $I = 1.7 \times 10^{25}$ photons . cm^{-2} . s^{-1}, dose 2×10^{18} photons . cm^{-2}).

3. Results and discussion

The low intensity ultraviolet irradiation (10^{18} photons . cm^{-2} . s^{-1}) of dilute ($\sim 10^{-4}$ M) aqueous solutions of thymidine, adenosine and 2′-deoxyadenosine results in but minor photoconversion of these nucleosides – the quantum yield is $\leqslant 10^{-5}$ [8]. This means that ultraviolet ΔA at 2×10^{18} photons . cm^{-2} are $\leqslant 0.1\%$.

The same doses of high intensity ultraviolet irradiation (1.7×10^{25} photons . cm^{-2} . s^{-1}) cause, however, a 15% decrease of thymidine solution A_{260} (fig.1). TLC analysis of the thus irradiated [2-^{14}C]thymidine solution revealed several radioactive reaction products (fig.2), one of which coincided with authentic thymine by its R_F values in solvent systems A, B and D and by its ultraviolet absorption spectra at pH 6.5 and 11.5. Several anisaldehyde-positive compounds were detected as well. One of them possessed the same R_F values in solvent system A, B and C and colouration as authentic 2-deoxyribose.

Thus high intensity ultraviolet irradiation brings about a number of consecutive and parallel transformations of thymidine, including scission of *N*-glycosidic bond, degradation of the thymine liberated (cf. [3]) as well as modification of the heterocyclic nucleoside moiety. Free thymine was shown to be 1.5-fold more susceptible to degradation in comparison to thymidine, from ultraviolet spectral data after ultraviolet irradiation with equal doses and intensities. Thymine accounts for ~30% of the total thymidine photoproducts thus indicating *N*-glycosidic bond scission and nucleoside heterocycle degradation proceed with similar efficiencies. Degradation of thymine within nucleoside is 1.5-times less efficient than in the free base.

The distribution patterns of radioactive and anisaldehyde-positive products formed upon high intensity

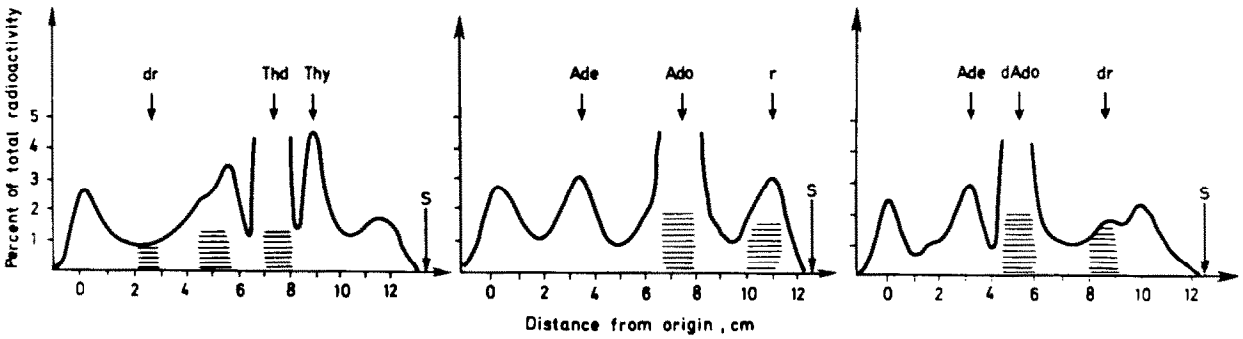

Fig.2. Distribution of radioactivity and anisaldehyde-positive components (hatched areas) upon TLC of irradiated solutions of thymidine (left, solvent A), adenosine (middle, solvent C) and 2′-deoxyadenosine (right, solvent C). Arrows indicate positions of thymidine (Thd), adenosine (Ado), 2′-deoxyadenosine (dAdo), thymine (Thy), adenine (Ade), ribose (r) and 2-deoxyribose (dr); S, solvent front. Irradiation conditions, see legend to fig.1.

ultraviolet and γ-irradiation of dilute aqueous solutions of [2-^{14}C]thymidine and those from 4-year autoradiolysis of solid nucleoside sample, are practically the same as shown by TLC analysis in solvent systems A and B. This implies the involvement of the 2-photon processes in the photoinduced degradation of thymidine, as takes place with thymine [3].

High intensity ultraviolet irradiation (1.7×10^{25} photons . cm^{-2} . s^{-1}) of adenosine and 2′-deoxyadenosine solutions brings about a decrease in A_{260} (fig.1) and formation of several reaction products (fig.2). Both nucleosides gave rise to adenine, identified from the coincidence of R_F values upon TLC in solvent systems C, E and F, as well as ultraviolet absorption spectra at pH 6.5 and 12, with those of an authentic sample. Besides, ribose and 2-deoxyribose were detected in the photoirradiated adenosine and 2′-deoxyadenosine solutions, respectively, from R_F values in the solvent systems mentioned and by the colour formation with the anisaldehyde reagent being the same as those of authentic sugars.

Analogous chromatographic patterns were observed upon TLC examination of autoradiolysis products of [U-^{14}C]adenosine and 2′-deoxy-[U-^{14}C]-adenosine. Thus the action of high intensity ultraviolet irradiation on adenine nucleosides causes cleavage of the *N*-glycosidic bond and degradation of both free and bound nucleic acid bases along with other photo-transformations.

Hence the photochemical conversions of adenine nucleosides as well as of thymidine at high ultraviolet irradiation intensities proceed through the higher electronic excited states arising by absorption of the second photon by the lowest excited states of the nucleosides.

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