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The photochemistry of purine components of nucleic acids. I. The efficiency of photolysis of adenine and guanine derivatives in aqueous solution

V.A. Ivanchenko, A.I. Titschenko, E.I. Budowsky+*, N.A. Simukova*, and N.S. Wulfson*

Scientific Research Institute of Organic Intermediate Products and Dyes, Moscow, USSR

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ABSTRACT.

It has been shown that the quantum yield of the photochemical conversion of adenine and the corresponding nucleosides and nucleoside 5'-phosphates in liquid (pH 5.6 and 2.0) and frozen aqueous solutions do not exceed 10^{-4}.

The quantum yield of the photoconversion of guanine-containing nucleosides and nucleoside 5'-phosphates in liquid aqueous solution (pH 5.6) after removal of oxygen by passing through nitrogen and in the frozen state do not exceed 0.3×10^{-4}. The quantum yield in oxygen-containing liquid aqueous solutions increase to 0.3×10^{-3}, i.e. to values commensurate with the quantum yield of pyrimidine photolysis.

INTRODUCTION

The UV-induced biological effects in cells, viruses and infectious nucleic acids arise mainly from the photochemical conversions of the nucleic bases. Hitherto it was the photochemical breakdown of the pyrimidines which had received the most attention largely because of their considerably larger quantum photolytic yields than those of the purines /1,2/. Recently, however, a number of papers have been published showing that in some cases the photo-efficiency of purine conversions can be commensurate with that of the pyrimidines. The photoinduced reactions of adenine and guanine occuring at the C(8) atom /3, 4, 5/. Pyrimidine-commensurate quantum yields are also observable in oligo- and poly-deoxyadenylic acids /6, 7/. Consequently data on the photochemical stability of purines in aqueous solution /8, 9/ require thorough investigation from both a kinetic and mechanistic standpoint. The results of such study may prove fundamental for elucidating the mechanisms of certain photobiological processes such

as UV-induced inactivation or mutation.

The present paper reports the results of an investigation into the photochemical stability of adenine and guanine derivatives in liquid and frozen aqueous solutions.

MATERIALS AND METHODS

Adenine (Ade) and guanine (Gua)-containing ribo- and deoxyribonucleosides, and nucleoside 5'-phosphates (pA, pG, dpA and dpG), kindly supplied by Dr. H.Maurer (Zellstofffabrik, DBR) were used without additional purification as they satisfied the usual criteria /10/.

The compounds under investigation in 0.35 ml portions of 10^{-3} M solutions in bidistilled water made up just before the run were placed in 10 cm polythene-stoppered quartz tubes (2.5 mm i.d.) and irradiated in a special chamber at 22° and -196° with the unfiltered light from a block of six 15w low pressure Hg vapor lamps emitting over 80% at 254 nm.

The quantum yields were evaluated by comparing their rates of photolysis with those of uridine /11/ under optically equivalent conditions. The incident light intensity was 16 $mE/cm^2/h$ The spectra of solution in the 200-350 nm range were taken on a Specord (Carl Zeiss, Jena) spectrophotometer. After irradiation the mixtures were subjected to thin layer cellulose chromatography using the following systems: n-butanol:ethanol: water:conc.NH_4OH (60:20:20:1,v/v); n-butanol:ethylmethylketone: water:conc. NH_4OH (40:30:20:10, v/v); isopropanol:water:glacial acetic acid (70:10:20, v/v), or on a Sephadex LH-20 column in ethanol:water (1:1, v/v). The mixtures from the irradiated adenylic and deoxyadenylic acids were chromatographed on a DEAE-Sephadex A-25 acetate column using a sodium acetate solution (pH 4.9; linear gradient 0.05→0.3 M) for elution. The course of the elution was monitored by means of UV-absorption at 254 nm. Spectra of the reaction mixtures and of the separated fractions were, as a rule, taken at pH 2, pH 5.6 and pH 9.

RESULTS AND DISCUSSION

The course and the rate of the photochemical conversion of the nucleic acid components depend upon the irradiation conditions.

The factors of greatest importance being the following:

1. The physical state of the system. Obviously if two molecules of the substance (as in the case of pyrimidine dimerization) are required to form the product, the frozen state which could fix the suitable orientation of the molecules should give rise to an increase in the quantum yield of the reaction. When each molecule independently undergoes photoconversion (as in the case of the photohydratation of pyrimidine) the reaction will not be affected by change in state.

2. The pH of the solution. Changes in the solution pH are accompanied by changes in the ratios of the ionic forms, reactivities of which usually differ rather widely.

3. The composition of the solution: the isotopic composition of the water, the presence of sensitizing agents, quenchers of triplets and radicals, etc. have a considerable effect on the reaction. Of great importance is oxygen, whose concentration in the water under usual conditions is $\sim 10^{-4}$M, which is often comparable to the concentration of the substance irradiated.

In view of this we irradiated the compounds in the liquid (22°C) and frozen (-196°C) states at pH 5.6 and 2.0 both in the presence of oxygen and after its removal by passing nitrogen through the reaction mixtures.

The photolysis products may differ from the initial compounds in their spectral characteristics. If such difference is great, one can judge of the nature and fate of the reaction already from the spectral changes in the reaction mixture occuring in the course of the irradiation. If it is not so great it can in most cases augmented by changing the pH of the solution while taking the spectrum . Finally, if the spectrum does not change during the photoreaction, the reaction does not take place or it is not accompanied by change in the chromophore, which in the case of nucleotides means that it is the sugar or phosphate residues which are affected. However, the products may then be revealed in chromatographing the reaction mixtures.

PHOTOLYSIS OF ADENINE DERIVATIVES

Fig. 1a shows the UV spectra of adenosine before and af-

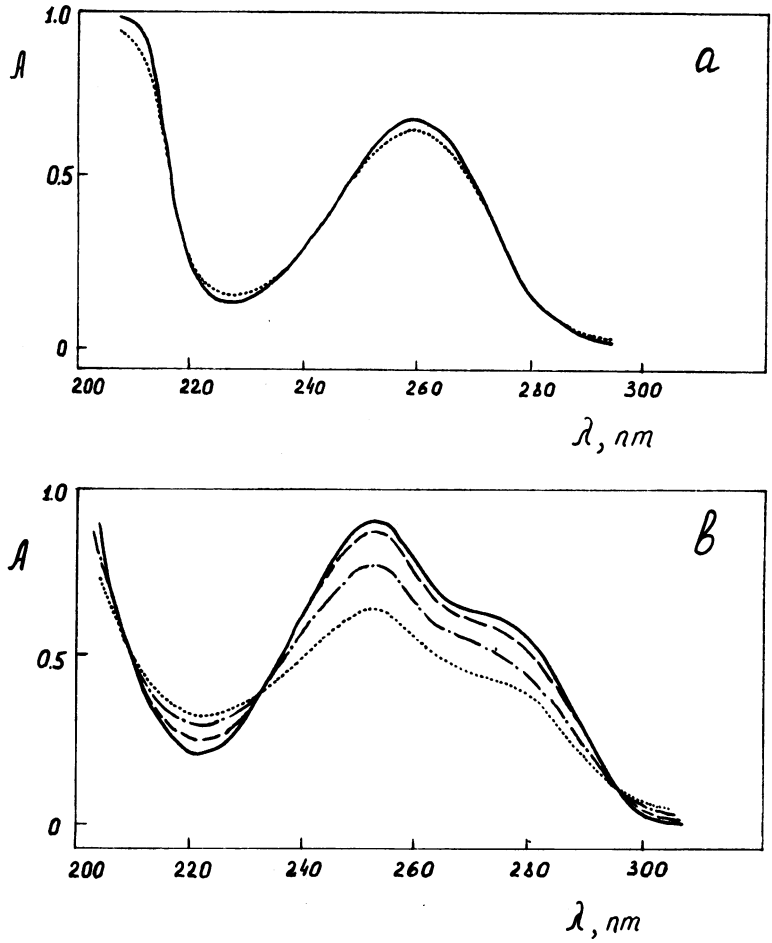

Fig. 1. Spectra of aqueous solutions of deoxyadenosine (a) and deoxyguanosine (b) after irradiation at 22°C (λ = 254 nm). The solid, dashed, dot-dashed and dotted lines refer to solutions before irradiation and after absorption of 350,600 and 1000 E/mole, respectively.

ter irradiation in a frozen aqueous solution. Table 1 presents the decrease in optical density at 260 nm after irradiation of the adenine derivatives.

It can be seen that bona fide spectral changes are detectable in all the derivatives investigated when the absorbed energy is 750 Einsteins per mole. The changes greatly depend on the pH of the solution being analyzed, evidencing the presence of U.V. absorbing products in the irradiated solutions. However, since these photoproducts were not further analyzed,

the changes in the spectra are only qualitative reflections of the photoconversion of the adenine nucleus.The quantum yields estimated from the spectral data are given in Table 2.

TABLE 1. Decrease in optical density at 260 nm of solutions of adenine derivatives after absorption of a dose of 750 E/mole (absolute error ±1.5%).

Irradiation conditions	pH of solution undergoing spectral measurement	Decrease in optical density (%)				
		Ade	A	dA	pA	pdA
Frozen aqueous solution, pH 5.6 -196°C	2	4	8	2.5	2	1.5
	5.6	3	2	4	2.5	1.5
	9	5	5	4	2.5	1.5
Liquid aqueous solution, pH 5.6 22°C	2	3	2	2	3	2
	5.6	2	1.5	2	3	2
	9	4	1.5	2	3	2
Liquid aqueous solution, pH 2 22°C	2	4	3	-	-	-
	5.6	3	2	-	-	-
	9	1.5	1.5	-	-	-

TABLE 2. Estimated quantum yields of photolysis of aqueous solutions of adenine derivatives (absolute error $\pm 0.20 \cdot 10^{-4}$)

Irradiation conditions	Quantum yield, $\times 10^4$				
	Ade	A	dA	pA	pdA
Frozen aqueous solution, pH 5.6, 196°C	0.6	0.9	0.5	0.3	0.2
Liquid aqueous solution, pH 5.6, 22°C	0.5	0.3	0.3	0.4	0.3
Liquid aqueous solution, pH 2, 22°C	0.5	0.4	-	-	-

It should be noted that no U.V. absorbing spots (other than those of the initial compounds) were found on thin-layer chromatography of irradiated Ade and dA, only an intense fluorescence being observable at the start of chromatogram. With Sephadex LH-20 the irradiated adenine solution (800 E/mole) yielded five new compounds absorbing at 254 nm emerged from the column before adenine, but their total optical density was less than 3% of that of the initial solution. After irradiation of pA and dpA solutions (800 E/mole) only initial compounds are observed in chromatography on DEAE Sephadex A25.

Thus UV-irradiation of adenine, adenosine, deoxyadenosine and the corresponding 5'-phosphates in both the liquid and frozen aqueous solutions had very little effect on these compounds. Even on assuming that some of the photoproducts could have had spectral characteristics only slightly different from the original compounds the quantum yields did not exceed 10^{-4}.

PHOTOLYSIS OF GUANINE DERIVATIVES

Because of low solubility of guanine only guanosine, deoxyguanosine and the corresponding 5'-phosphates were irradiated. Irradiation of both the frozen and liquid aqueous solutions through which nitrogen had been preliminary passed brought about spectral changes that did not exceed those for the adenine derivatives. However, when the solutions not purified of oxygen were irradiated appreciable changes in the spectra took place (Fig.1, Table 3). Chromatography on Sephadex LH-20 of the irradiated oxygen-containing deoxyguanosine solution (600 E/mole) revealed a number of compounds, some of which displayed marked absorption at $\lambda \geqslant 250$ nm as well as the initial substance. The structural determination of these products which is now in progress will aid in the elucidation of the reaction mechanism. But already stage it is quite apparent that the presence of oxygen is a prerequisite for the reaction to take place.

The photochemical conversions of guanine derivatives are not stimulated by the heavy metal ions, because passage of the solution through the Chelex-100 bed does not affect the

TABLE 3. Decrease in optical density at 260 nm of solutions of guanine derivatives after absorption of a dose of 600 E/mole (absolute error ± 1.5%)

Irradiation conditions	pH of solution undergoing spectral measurement	Decrease in optical density (%)			
		G	dG	pG	pdG
Liquid aqueous solution, pH 5.6, 22°C	2	14	18	8	12
	5.6	9	14	5	11
	9	7	18	7	12
Liquid aqueous solution, pH 5.6, 22°C, irradiated after passing through nitrogen for 60 min	2	1.5	1.5	1.5	2
	5.6	2	2	2	3
	9	1.5	3	2	1.5
Frozen aqueous solution, pH 5.6, -196°C	2	1.5	1.5	1.5	1.5
	5.6	1.5	1.5	1.5	2
	9	1.5	1.5	1.5	1.5

TABLE 4. Estimated quantum yields of photolysis of aqueous solutions of guanosine derivatives (absolute error $\pm 0.25 \cdot 10^{-4}$)

Irradiation conditions	Quantum yields $\times 10^4$			
	G	dG	pG	pdG
Liquid aqueous solution, pH 5.6, 22°C	2.2	3	1.2	1.8
Liquid aqueous solution, pH 5.6, 22°C, irradiated after passing through nitrogen for 60 min	0.30	0.5	0.3	0.5
Frozen aqueous solution, pH 5.6, -196°C	0.25	0.25	0.25	0.3

rate of UV-induced alterations of deoxyguanosine.

It should be stressed that since the photolytic products are able to absorb radiant energy at $\lambda \geqslant 250$ nm they may un-

dergo further conversions under the conditions of the irradiation experiment. Moreover, their formation is not accompanied by a proportional reduction of the absorption at 250-280 nm by the reaction mixture. Nevertheless, the quantum yields of the photoconversions of guanine derivatives in the presence of oxygen (see Table 4) as estimated by the decrease in the optical density attains a value of 0.3×10^{-3}, which is commensurate with the quantum yields of the photoconversion of pyrimidine in nucleic acids /12/.

It is noteworthy that the results obtained are consistent with the increase in photolability of purine derivatives caused by substitution at C(2) /8,9/. At the same time our data are in disagreement with reported earlier that oxygen inhibits the photochemical conversion of purines substituted at C(2) and C(6).

Since oxygen is usually not removed when irradiating biological objects (cells, viruses, etc.) it is evident that photoconversion of the guanosine residues in polynucleotides might be one of the essential factors in the biological effects of UV-irradiation.

*Shemyakin Institute of Bioorganic Chemistry Ac. Sci. USSR, Moscow 117312, ul Vavilova 32, USSR
+ To whom correspondence should be addressed

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