The photochemistry of purine components of nucleic acids. II. Photolysis of deoxyguanosine

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Received 14 February 1977

ABSTRACT

UV-irradiation ($\lambda = 254$ nm) of liquid aqueous solutions of deoxyguanosine in the presence of oxygen at pH < 7 causes an intensive degradation of nucleoside. The quantum yield estimated from A_{254} decrease for the reaction mixture was found to be 1.5×10^{-4} at doses to 150 E/mole. The rate of A_{254} decrease was found to grow with increasing doses.

The structures of the products isolated from the reaction mixture after irradiation suggest that one of the ways of deoxyguanosine degradation is a breakdown of a purine cycle without splitting of N-glycoside bond. Simultaneously another type of photoinduced modification of guanine nucleus takes place, which is followed by appearance of free 2-deoxyribose. Deoxyguanosine degradation in both directions is kinetically one-step process proceeding with comparable quantum yields of $\sim 1 \times 10^{-4}$.

INTRODUCTION

Biological consequences of UV-irradiation in cells and viruses are due to photochemical changes in genetic materials. Practically all the constituents of nucleic acids can undergo changes on UV-irradiation ($\lambda = 254$ nm). In addition to the formation of pyrimidine dimers of different types but with comparable quantum yields photohydration of cytosine and uracil nuclei [1,2], degradation of deoxyadenosine [3,4] and deoxyguanosine [5] units as well as formation of polynucleotides-protein linkings [1,6,7,8] can take place. The contribution of each of these photochemical reactions to the biological consequences of UV-irradiation has not been definitely determined yet. The data available indicate the formation of thymine dimers is not the main cause of mutation and inactivation of genetic materials upon UV-irradiation.

Thus inactivation of RNA-containing phages essentially results from photohydration of a uracil nucleus [9,10], while UV-induced mutation in DNA-containing phage is considerably due to photohydration of a cytosine nucleus [11]. A direct quantitative determination of all types of photodamages in polynucleotides and nucleoproteins is impossible at present. To elucidate the role of each of the photoproducts in biological consequences upon UV-irradiation investigations of mechanisms and kinetics of photoreactions in which all the constituents of nucleic acids take part, as well as studies on the structures and properties of both primary photoproducts and those of their subsequent (dark) conversions are quite necessary.

In the present paper the results of our investigations on the substances produced by UV-irradiation of deoxyguanosine in aerated liquid aqueous solutions are presented.

MATERIALS AND METHODS

Deoxyguanosine (dG) purchased from Calbiochem was purified by chromatography on a Sephadex LH-20 column (see below). The UV-irradiation source was two 15 w low-pressure mercury lamps disposed inside two consecutively-connected flow quartz cylindric reactors (the thickness of the irradiated layer was ~ 0.16 cm, the diameter ~ 4 cm, the length ~ 32 cm; the volume of the two reactors ~ 130 ml). The intensity of the light beam (at 254 nm) passing through the reactors was $3 \cdot 10^{-2}$ Einstein/hr as estimated from the initial rate of uracil photolysis [12].

While being irradiated deoxyguanosine solution (~ 200 ml) was pumped with a micropump into the consecutively connected reactors from an open cylinder; after the last of them was filled up, the solution flew down into the cylinder. Thus a desirable circulation rate (~ 1500 ml/hr) and a constant contact of the reaction mixture with an atmospheric air during irradiation were achieved. Irradiation was carried out at 25°C . Deoxyguanosine solutions (pH 5.6) were prepared using bidistilled water directly before irradiation, their concentration being 10^{-3}M . After irradiation the reaction mixture

was evaporated to dryness on a film evaporator, with the procedure usually lasting 1-2 hours. A resulting residue (~60 mg) was dissolved in 1-3 ml of an eluent and then it applied onto Sephadex LH-20 column (2.5x185 cm). The eluent-ethanol-water (1:1, v/v), the elution rate was 15 ml/hr. The separation process was followed by UV-absorption at 235 nm, 254 nm and 300 nm as well as by absorption at 595 nm which appeared when the fractions were subjected to a Dische reaction to detect 2-deoxyribose [13]. After column chromatographing the reaction mixture fractions were controlled also by TLC on cellulose in the following systems: n-butanol-methanol-water (7:7:6, v/v) and n-butanol-water (86:14, v/v). An ultrachemscope was used to detect the positions of UV-absorbing spots on the thin layer. Elemental analysis (determination of C/N ratio) was kindly carried out by Dr. M.N.Chumachenko from our Institute. A VSU-2 spectrophotometer (Carl Zeiss, Jena) was used to obtain spectra of reaction mixtures and fractions after chromatographic separation. Mass-spectra were recorded on a LKB 2091 mass-spectrometer, with direct inlet system at 100°, 150° and 260°C and ionizing electron energies of 12 and 70 ev. PMR spectra of solutions in D₂O and DMSO (~25 mg/ml) were recorded on a Varian spectrometer XL-100 at 100 MHz. The TMS signal was taken as a reference. To assign the proton signal experiments were carried out to selectively resolve spin-spin interaction (double nuclear magnetic resonance).

RESULTS AND DISCUSSION

To preparatively separate the reaction mixtures after deoxyguanosine is irradiated chromatography on LH-20 Sephadex (hydroxypropylated Sephadex G-25 [14]) was used. A 1:1 mixture (v/v) of ethanol and water was a mobile phase since most of nucleosides are soluble in it. The compositions of free and absorbed phase differ considerably because of lipophilic character of Sephadex LH-20 [15] and separation of the components should proceed via mechanisms both of exclusion and partition chromatography. A wide column (i.d. 2.5 cm) was used to prevent the wall effect. The elution rate

primarily depended on the permeability of a column and was $\sim 3.2 \text{ ml/cm}^2/\text{hr}$ in our experiments. Decreasing of the elution rate resulted in only insignificant improvement of separation. Temperature deviations of the column exceeding $\pm 1^\circ$ deteriorated the quality and reproducibility of separation. Shown in Fig.1 is a pattern of chromatographic separation of 6 nucleosides. It can be seen from the pattern that nucleosides are eluted as narrow peaks which can be regarded as an evidence of sufficiently high efficiency of the method. The number of theoretical plates estimated by a conventional method [16] was about 6500 per the column, i.e. not less than 35 per 1 cm of its length. It should be noted that separation was carried out in a neutral non-buffered ethanol-water mixture at 22°C so that even unstable compounds could be isolated and the separation process could be followed either by optical density at $> 220 \text{ nm}$ or by colour reactions.

Fig.1. The chromatography on Sephadex LH-20 column of inosine (I), uridine (U), 5-hydroxyuridine (ho^5U), guanosine (G), adenosine (A) and 4-thiouridine (s^4U) in the system ethanol-water (1:1, v/v).

We could separate the mixture resulting from irradiation of aqueous deoxyguanosine solutions into a series of satisfactorily resolved peaks (by A^{235} , Fig. 2a) by chromatography on Sephadex LH-20. However, as it follows from the spectral data (the ratio A^{235}/A^{254} , Fig.2a) peaks 1, 2, 4, 5 and 8 contain mixtures of substances. The TLC data (Table I) lead to a similar conclusion. Non-homogeneity of the peaks

Fig.2. The chromatography on Sephadex LH-20 of reaction mixture resulting from UV-irradiation of liquid aqueous solutions of deoxyguanosine after absorbing 300 E/M (a) and of the peak 6 compound (see a) after incubating at 22°C for 3 hours and subsequent evaporating on a film evaporator to 0.5 ml (b). Solid, dotted and pointed lines represent optical densities at 235 nm, 254 nm and 595 nm (after a Dische reaction in the last case).

may be due both to insufficient resolution ability of column chromatography and to new conversions which reaction products undergo during chromatography, fraction concentration or preparation storage. Indeed, some of the reaction products undergo further changes upon storage, even lyophilized prepa-

Table 1. R_f values for the products produced by UV-irradiation of an aqueous deoxyguanosine solution.

peak N (see Fig.1)	n-butanol:water (86:14, v/v)	n-butanol:water:met- hanol (7:6:7, v/v)
1	0-0.02	-
2	0.02-0.04	-
3	0.10 ^{***}	0.48 ^{***}
4	0.04	0.25
	0.10	0.40
	0.20	
5	0.44 ^{***}	0.60 ^{***}
5'	0.20 ^{***}	0.55 ^{***}
6	0.10	0.48 ^{***}
	0.16 ^{***}	0.50 ^{***}
	0.25	0.70
6	0.10	0.48
	0.16	0.50
	0.26 ^{***}	0.70 ^{***}
7	0.30 ^{***}	0.41 ^{***}
8	0.12	0-0.15
	0.20 ^{***}	0.33 ^{***}

Unless otherwise denoted the products were detected by their absorption at $\lambda = 254$ nm.

^{***}) detected by Dische reaction

^{***}) the main compound in the peak.

rations do. After concentration of the solution and keeping it for 3 hours in an aqueous solution at 22°C about a half of the substance of peak 6 converts into products whose chromatographic mobilities coincide with those of peaks 3 and 4 (Fig.2b, Table 1). The substances of peaks 1 and 2 are almost completely lost upon their subsequent rechromatography.

Only substances of peaks 3,5' (see below) and 6 of all chromatographically separable on LH-20 are stable enough to be isolated and their structures can be studied.

As it can be seen from Fig.2a only one of the products in peak 6 gives a considerable absorption in the long-wave region of the spectrum in addition to that of the starting compound (peak 7). Hence, this product contains an aromatic fragment. Only substances of peaks 3, 6 and of the left-hand part of peak 5 (peak 5', Fig. 2a, the dotted line) in addition to the starting deoxyguanosine are shown to contain deoxyribose. It follows then that the compounds of peaks 1, 2, 4, 8 and of the right-hand part of peak 5 contain only degradation products of the guanine nucleus.

Those fragments of the nucleoside unit which remain in

a polynucleotide chain after irradiation, i.e. those which contain deoxyribose (the substances of peaks 3,5' and 6) are, evidently, of particular interest for photobiology. Stable substances of peaks 3 and 5' were isolated in pure form by rechromatography on Sephadex LH20 column.

Compound 5' was identified as 2-deoxyribose from a coincidence of its mass-spectra with those in literature [17,18], its chromatographic mobility in a number of systems and Dische reaction.

The UV-spectrum of the peak 3 compound ($\lambda_{\text{max}}=235 \text{ nm}$) evidenced the absence of aromatic structure and did not change with pH in the pH range from 2 to 10. The presence of the deoxyribose residue in the compound was supported by PMR data (Fig.3) in addition to Dische reaction. The PMR spectrum of D_2O solution contained proton signals of the deoxyribose residue hydrogens (cf. [19]). The signal at 4.0ppm (Fig.3a) resembled to more than two protons (~ 2.4), which evidenced the presence in the peak 3 compound of one more relatively exchangeable proton (most probably at C5, see Scheme below). The PMR spectrum of the peak 3 compound in deuterodimethyl sulfoxide (Fig.3b) shows a doublet at $\sim 8.05 \text{ ppm}$ (one proton) and a broad signal centered at $\sim 7.6 \text{ ppm}$ (four protons) in addition to the proton signal from the deoxyribose residue. The proton-proton suppression of spin-spin coupling experiments and analysis of PMR spectra available in literature [20] indicated the signal at $\sim 8.05 \text{ ppm}$ to be due to the proton at the nitrogen in the amide group directly connected with C1 in the deoxyribose.

Elemental analysis of the peak 3 compound gave the ratio $\text{N/C} = 0.33 \pm 0.01$. Thus the compound should contain C_9N_3 (the ratio for C_6N_2 disagrees with the PMR data) taking the presence of the deoxyribose residue and elemental composition of the starting deoxyguanosine into account.

Mass-spectra of the peak 3 compound are given in Fig.4. Although the molecular ion peak does not appear in the spectra, the results of fragmentation together with elemental analysis, UV- and NMR-data allow us to suggest the following structure for this compound:

The main compound of peak 6 contains a deoxyribose residue as it is evident from PMR data. This compound has characteristic UV spectrum in a neutral aqueous solution with $\lambda_{\text{max}} = 253 \text{ nm}$. The pK values determined by spectrophotometric titration were ~ 3.5 and 9.5 .

Elemental analysis of the peak 6 compound and its ability to turn into the peak 3 compound spontaneously allowed to ascribe the composition $C_{10}N_4$ to it. Thus, deoxyguanosine conversion into the peak 6 compound can be said to proceed via a loss of one nitrogen atom. This conversion does not however come to a simple deamination since deoxyxanthosine differs greatly from the peak 6 compound in its UV spectrum (Fig.5a, cf. [2]) and stability. As it has been stated above the peak 6 compound undergoes further conversion in the dark even in neutral aqueous solutions, which results in a decrease of the long-wave absorption. The rates of the conversions (measured from the changes in the spectra) at pH2 and pH 10 (Fig.5b) are by almost one order of magnitude higher than those in neutral solution and were found to be $6 \times 10^{-3} \text{ min}^{-1}$ and $2.5 \times 10^{-3} \text{ min}^{-1}$, respectively. These data suggested that the photo-induced deoxyguanosine conversion into the peak 6 compound should be due not only to a loss of one nitrogen but to some alteration(s) of the heterocycle without loss of aromaticity.

Fig.3. The PMR spectra of the peak 3 compound in D_2O (a) and in deuteriodimethyl sulfoxide (b).

Fig.4. The mass-spectra of the peak 3 compound $260^{\circ}C$, ionizing electron energies 70 eV (a) and 12 eV (b).

Fig.5. a) UV-spectra of the peak 6 compound at pH 5.6 (solid line) and pH 2 (dotted line) and pH 10 (broken dotted line); the spectra were normalized by deoxyribose content. b) Alterations in the UV-spectra of the peak 6 compound upon its incubation in aqueous solutions at 25°C. The figures on the lines represent durations (minutes) of the incubation.

Only further conversion into the peak 3 compound resulted in the loss of aromaticity and of the C_1N_1 fragment.

Thus, it can be assumed that one of the main ways of deoxyguanosine photodestruction is alteration of an aglycone without N-glycoside bond splitting when liquid aqueous solutions ($pH < 7$) are irradiated by doses up to 600 E/mole in the presence of oxygen. This is kinetically a one-step route because quantities of the peak 3 and 6 substances increase linearly during irradiation (Fig.6). The linear character of the peaks 3 and 6 accumulation in spite of consecutive $dG \rightarrow 6 \rightarrow 3$ conversion can probably be accounted for by that the last (dark) step proceeds during long term isolation of photoproducts rather than during irradiation.

Fig.6. UV-induced changes in the number of the reaction products of deoxyguanosine detected by absorption at 235 nm as percentage of the A^{235} of the starting solution. The figures on the lines correspond to the peak numbers in Fig.2. The dotted line represents a change in the optical density of the reaction mixture at 254 nm.

The quantity of free deoxyribose also increases linearly in the reaction mixture when deoxyguanosine is irradiated (Table 2). The N-glycoside bond in compounds 6 and 3 was found to be stable under the reaction conditions and isolation of the products. Hence, free deoxyribose is not produced from these two compounds, and the efficiency of the two ways in photoconversions of deoxyguanosine (viz., splitting of the N-glycoside bond and formation of compounds 6 and 3) should be practically equal (Table 2). It is necessary to note that splitting of the N-glycoside bond is evidently due to alterations in the aglycone since no guanine was detected in the reaction mixtures after irradiation of deoxyguanosine.

As it can be seen from Fig. 6 the A^{254} value for the reaction mixture decreases linearly at doses to 150 E/M, while the rate of this decrease starts to grow upon further irradiation. The rate of substance accumulation in peaks 5 and 8 also increases within this range of doses. These findings may be regarded as reflecting some processes of photodegradation of deoxyguanosine which become apparent at high doses.

It must be noted that photodestruction of dG anion proceeds by one order of magnitude more rapid than that of a neutral molecule and results in quite a different set of compounds, though it also requires that the oxygen should be present (will be published separately). In some respect the conversions, discussed above, are similar to the photodynamic effect [21, 22] - in both cases the presence of oxygen

Table 2. The extent of deoxyguanosine conversion (determined by the A^{254} of the reaction mixture) and yields of some photoproducts (determined by deoxyribose content) resulting from UV-irradiation ($\lambda = 254$ nm) of an aqueous deoxyguanosine solution in the presence of oxygen (pH=5.6)

Compound	Dose, E/mole		
	150	300	450
Deoxyguanosine	$2 \pm 0.5\%$	$6 \pm 0.5\%$	$16 \pm 0.5\%$
peak 3	$0.4 \pm 0.1\%$	$1 \pm 0.2\%$	$1.6 \pm 0.2\%$
peak 6	$0.7 \pm 0.2\%$	$1.6 \pm 0.2\%$	$2.6 \pm 0.3\%$
peak 5 ¹	-	$2.4 \pm 0.3\%$	$4.0 \pm 0.5\%$

is a prerequisite and increase of pH result in the increase of the destruction rate. But the real mechanism of primary photoreactions, taking place upon irradiation of the oxygen-containing aqueous liquid solution of dG, still remains to be clarified. It is possible that the reported recently significant reversible alteration of the spectrum under UV-irradiation of GMP solution in ethylene-glycol-water at -110° [23] reflects formation of the primary product of the processes discussed above. However the available data are sufficient to consider biological consequences of UV-irradiation in cells and viruses. First of all a pronounced effect of oxygen on the quantum yield for degradation of the deoxyguanosine unit [5] should be mentioned. The quantum yield for this photoreaction in the presence of oxygen even at pH below 7 is only by one or two orders lower than those for photoconversions of pyrimidines [5]. As it has mentioned above further increase of pH is accompanied by a sharp acceleration of photoinduced decomposition of dG. It is well established that a reversion of cytosine photohydrates [24] and enzymic decomposition of cyclobutane [25] and non-cyclobutane dimers of pyrimidines [26] proceed with ease, thus considerably reducing biological consequences of photo-induced modification in pyrimidine units of DNA. It may be assumed that conversions of deoxyadenosine [3, 4] and deoxyguanosine (see ref.[5] and above) units, though proceeding with lower quantum yields, are the essential cause of UV-induced mutations in and inactivation of cells and viruses. It should be noted that not only photo-induced and dark conversions of the deoxyguanosine unit result in modifying of the guanine nucleus but in splitting of N-glycoside bond as well, and the latter is known [2] to facilitate a breakdown of polynucleotide chain. The last two types of DNA damaging are much more essential in genetic processes than alterations in the structure of a base. This also supports the above assumption concerning the consequences of photo-induced conversions in deoxyguanosine units.

REFERENCES

1. Smith, K.C. and Hanawalt, P.S. (1969) Molecular Photobiology, Ac. Press, N.Y.-London.
2. Kochetkov, N.K., and Budowsky, E.I. eds., (1972) Organic Chemistry of Nucleic Acids, Part B, Chapter 12, Plenum Press, London-N.Y.
3. Pörschke, D. (1973) Proc. Nat. Ac. Sci. USA, 70, 2683-2686.
4. Pörschke, D. (1973) J. Am. Chem. Soc. 95, 8440-8446.
5. Ivanchenko, V.A., Titschenko, A.I., Budowsky, E.I., Simukova, N.A. and Wulfson, N.S. (1975) Nucleic Acids Research, 2, 1365-1373.
6. Budowsky, E.I., Simukova, N.A., Turchinsky, M.F., Boni, I.V., and Skoblov, Yu.M. (1976) Nucleic Acids Research, 3, 261-276.
7. Simukova, N.A. and Budowsky, E.I. (1974) FEBS Letters 38, 299-303.
8. Aging, Carcinogenesis and Radiation Biology (1976), Smith, K.C. ed., Plenum Press, N.Y.-London.
9. Remsen, J.F., Miller, N. and Cerutti, P.A. (1971), Biochemistry 10, 524-529.
10. Remsen, J.F. and Cerutti, P.A. (1972) Bioch. Biophys. res. commun. 48, 430-436.
11. Krivisky, A.S., Esipova, V.V. and Budowsky, E.I. (1975) Genetika (in Russian), 9, No. 9, 28-103.
12. Wierzychowski, K.L. and Shugar, D. (1959) Acta Biochim. Polon. 6, 313.
13. Dische, Z. (1954) in The Nucleic Acids (Chargaf and Davidson, eds.), Academic Press, New York.
14. Sephadex LH-20, Technical Booklet (1972), Pharmacia, Uppsala.
15. Sjövall, J., Nyström, E. and Haanti, E. (1968) Advan. Chromatog. 6, 119-170.
16. Martin, A.J.P., Syngé, R.L.M. (1941) Biochem. J., 35, 1358.
17. Biemann, K. (1962) Mass Spectrometry, McGraw-Hill Book Company, Inc., New York.
18. Adrien, A. (1973) in Synthetic Procedures in Nucleic Acid Chemistry (W.W.Zorbach and R.S.Tipson, eds.) Volume 2, John Wiley and Sons, Inc.
19. Townsend, L.B. (1973) in Synthetic Procedures in Nucleic Acid Chemistry (W.W.Zorbach and R.S.Tipson, eds.) Volume 2, John Wiley and Sons, Inc.
20. Bovey, F.A. (1972) High resolution NMR of macromolecules, Academic Press, New York.
21. Simon, M.I. and van Veenakis, H. (1963) Arch. Biochem. Biophys., 105, 197-206.
22. Waskell, L.A., Sastry, K.S. and Gordon, M.P. (1965). Biochim. Biophys. Acta 129, 49-53.
23. Morgan, J.P. and Callis, P.R. (1976) Photochem. Photobiol., 23, 131-134.
24. DeBoer, G., Klinghoffer, O. and Johns, H.E. (1970) Bioch. Biophys. Acta, 213, 253-268.
25. Setlow, J.K. (1966) Radiation Res. Suppl., 6, 141.
26. Munokata, N. and Rupert, C.S. (1974) Molec. Gen. Genet., 130, 239-250.