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LASER-INDUCED PHOTOIONIZATION AND SINGLE-STRAND BREAK FORMATION FOR POLYNUCLEOTIDES AND SINGLE-STRANDED DNA IN AQUEOUS SOLUTION: MODEL STUDIES FOR THE DIRECT EFFECT OF HIGH ENERGY RADIATION ON DNA

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Summary

Quantum yields for the formation of hydrated electrons (Φ_{e^-}) and single-strand breaks (Φ_{ssb}) were measured for polyadenylic acid (poly A), polycytidylic acid (poly C), polyuridylic acid (poly U) and single-stranded (ss) DNA in deoxygenated aqueous solution at room temperature upon nanosecond laser excitation at 248 nm. The values $\Phi_{e^-} = (1.7 - 3) \times 10^{-2}$ and $\Phi_{ssb} = (0.5 - 5) \times 10^{-4}$ at intensities of $(0.5 - 1.5) \times 10^7$ W cm^{-2} were obtained. The results show that base radical cations of these polynucleotides and of ssDNA lead to ssb formation. The large difference between Φ_{e^-} and Φ_{ssb} (a factor of 60 - 380) is explained as being the result of back reactions of geminate pairs of radicals and/or radical ions. Conductivity changes after the laser pulse, representing ssb formation, reveal a fast increase and a consecutive slow increase for all nucleic acids studied. The activation energies and pre-exponential factors, calculated from the rate constant k_{obs} of the slow conductivity increase at pH 7.3, are reported. Dithiothreitol (DTT) inhibits ssb formation of poly C (rate constant $k_{DTT} = 1.7 \times 10^6$ M^{-1} s^{-1}) and poly U whereas for poly A and ssDNA a smaller effect was found ($k_{DTT} < 0.7 \times 10^6$ M^{-1} s^{-1}). The pH dependence of k_{obs} is very similar for poly A and ssDNA, but different from that of poly C and poly U. This indicates that the slow strand break formation in ssDNA upon laser excitation is related to the purine moiety.

1. Introduction

Two quantum absorption processes of DNA and polynucleotides upon laser excitation have become of increasing interest because they allow experimental access to the so-called direct effect of high energy irradiation on DNA in aqueous solution at room temperature. Biphotonic excitation of the chromophore of the nucleic acid bases (maxima around 260 nm) has been carried out by high power picosecond and nanosecond laser pulses [1 - 8]. Nikogosyan *et al.* [3, 4] have shown for isolated nucleotide bases that upon picosecond excitation the second photon is absorbed by the excited singlet

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state S_1 whereas upon nanosecond excitation the second photon is absorbed mainly by the triplet state. This was concluded from the short lifetime of the S_1 state (a few picoseconds [3, 9]) and the low intersystem crossing quantum yield [3, 10 - 12] (*e.g.* $\Phi_{isc} \approx 2 \times 10^{-3}$ for adenine [3]). Recently the mechanism of excitation energy transfer along the polynucleotide chain has been investigated and results for single-strand break (ssb) formation in polyuridylic acid (poly U), pBR 322 plasmid DNA, phage DNA and RNA upon biphotonic picosecond laser excitation have been published [6]. For the single-stranded poly U or TMV RNA the effective energy transfer distance corresponds to 1 - 2 nucleotides whereas in double-stranded (ds) DNA or RNA the energy may migrate 50 - 170 nucleotides. For TMV RNA a factor of 250 was found for the ratio of Φ_{e^-} to Φ_{ssb} (Φ_{e^-}, quantum yield for the formation of hydrated electrons; Φ_{ssb}, quantum yield for the formation of single-strand breaks).

In a preceding paper we studied the chemical steps leading to ssb formation of poly U in argon-saturated aqueous solution using time-resolved electrical conductivity measurements following a 248 nm laser pulse [7]. A fast component and a slow component of the conductivity increase were observed, at least the latter reflecting ssb formation. The rate-determining step of the slow component of the conductivity increase has been shown to be identical with that for OH-radical-induced ssb formation [7, 13]. This observation allowed a chemical mechanism to be postulated for the laser-induced ssb formation of poly U. The cleavage of the sugar phosphate bond has been interpreted as being the rate-determining step [13]. However, recent results for OH-radical-induced ssb formation have indicated that the rate-determining step is the hydrogen-atom abstraction from the sugar moiety by base radicals [14]. The slow conductivity increase upon photoionization of poly U is therefore now reinterpreted as being determined by the same step.

The goal of the present paper is to extend the mechanistic studies of strand break formation to polycytidylic acid (poly C), polyadenylic acid (poly A), and single-stranded DNA (ssDNA). Φ_{ssb} and Φ_{e^-} and conductivity changes were measured. The results indicate that not only the radical cations from uracil but also those from cytidine and adenine can initiate strand breaks. The quantum yields are compared with published data for γ radiolysis and the difference between Φ_{e^-} and Φ_{ssb} (a factor of 60 - 380) is discussed. To account for this difference we propose a combination of geminate pairs of radicals and/or radical ions. This conclusion is supported by the value of the lifetime of the hydrated electron and by the effect of radical or electron scavengers on Φ_{ssb}. Furthermore, it is shown that the conductivity increase of ssDNA upon laser excitation exhibits a pH dependence very similar to that of poly A (a purine-containing polynucleotide), whereas the OH-radical-induced conductivity increase is characterized by behaviour similar to that of poly U or that of poly C (pyrimidine-containing polynucleotides); this is evidence that ssb formation in ssDNA upon photoionization is related to the purine moiety.

2. Experimental details

2.1. Materials

Poly A, poly C and poly U as their potassium salts and dithiothreitol (DTT) were obtained from Boehringer (Mannheim). Another poly U sample was obtained from P L Biochemicals (Milwaukee), and DNA (calf thymus) was purchased from Serva (Heidelberg) or from Merck (Darmstadt). *t*-Butanol (Merck, Darmstadt) was purified by zone refining. 2-Chloroethanol (Merck, Darmstadt) was purified by distillation. The DNA samples were denaturated by heating (up to 90 °C for 20 min) prior to use and rapid cooling in an ice bath. For the determination of Φ_{e^-} the nucleic acid solutions were membrane filtrated (Amicon UM 10) to remove low molecular weight components which might be present; the solutions were concentrated by a factor of 10 and afterwards rediluted with water. Deoxygenation was achieved by purging the solutions with argon (purity, better than 99.998%) or N_2O (oxygen removed by the use of an Oxisorb column) for 60 min. Water was purified by a millipore system [7] or by distillation (six times). Optical density values OD refer to λ_{max} (near 260 nm) in a 1 cm cell.

2.2. Conductivity measurements

The 248 nm line of an excimer laser (EMG 200, Lambda Physik; pulse width, 20 ns) and the conductivity apparatus were used as described previously [7]. Data analysis was improved by use of a transient digitizer (Tektronix 390 AD) which was connected to a computer (PDP 11/34). The high frequency AC bridge was operated at 300 kHz. The laser beam was limited by an aperture (ϕ = 5 mm).

In order to enhance the conductivity increase $\Delta\kappa$ by decreasing the molecular weight, the solutions for the conductivity experiments were in some cases γ preirradiated with a ^{60}Co γ source after N_2O saturation and were subsequently saturated with the appropriate gas. For ssDNA the initial weight-averaged molecular weight $\bar{M}_w = 2 \times 10^6$ g mol^{-1} was reduced to about 5×10^4 g mol^{-1} by a preirradiation dose of 2 - 4 krad. For the subsequent laser excitation (laser energy E_L = 30 mJ) the solution was adjusted to OD = 2. Without preirradiation the same kinetics were found but values for $\Delta\kappa$ were smaller than with preirradiation even at E_L = 80 mJ. For poly A the applied dose (1 krad) reduced the initial $\bar{M}_w$ from 8×10^5 g mol^{-1} to 6×10^4 g mol^{-1}. The laser excitation was performed at OD = 1 with E_L = 70 mJ. Poly C was preirradiated with 0.2 krad (initial $\bar{M}_w = 8 \times 10^5$ g mol^{-1}, final $\bar{M}_w = 1 \times 10^5$ g mol^{-1}) and the laser excitation was performed at OD = 1 and E_L = 30 - 45 mJ. It was checked that preirradiation had no effect on the kinetics at 23 °C and at different pH values for poly A and poly C.

2.3. Effective absorption coefficient

The dependence of the effective absorption coefficient ϵ_{eff} on the laser intensity I_L was calculated using the equation

$$\epsilon_{\text{eff}} = \frac{\log(E_0/E)}{cl} \tag{1}$$

E_0 and E are the energies of the transmitted light when the cell (path length $l = 1$ cm) was filled with either the solvent alone or the nucleic acid solution (at a concentration c) respectively. The absolute and relative energies were measured with a calibrated energy detector (14 NO, Laser Instr.) and a radiometer (model 580, EG+G). The quartz cell was shielded by an aperture ($\phi = 5$ mm) and about 10% of the incident laser energy was reflected onto the radiometer by a quartz plate placed in front of the aperture. The OD at 248 nm was kept below 0.6 in order to minimize a possible differential change in ϵ_{eff}. The incident laser intensity I_L was derived from the incident laser energy (behind the cell entrance window, taking into account 5% reflection on the quartz window), the laser pulse width and the irradiated area. The nucleotide concentration was obtained from OD values using the following absorption coefficients (at λ_{max}): poly U, 9.7×10^3 M^{-1} cm^{-1} [15]; poly C, 7.0×10^3 M^{-1} cm^{-1} [16]; poly A, 10.1×10^3 M^{-1} cm^{-1}; ssDNA, 8.7×10^3 M^{-1} cm^{-1} [17].

2.4. Quantum yield of hydrated electron formation

The hydrated electrons were detected by transient absorption spectroscopy [18] at 715 nm with nucleic acid concentrations corresponding to OD = 0.5 - 2 at 248 nm. The electron concentration in the detectable volume ($V = 0.04$ cm^3) was obtained from the maximum OD during the laser pulse using $\epsilon_{715} = 1.8 \times 10^4$ M^{-1} cm^{-1} [19]. The hydrated electrons were characterized by their absorption spectrum ($\lambda_{\text{max}} \approx 715$ nm [19]) and by the effects of quenchers (N_2O, O_2 and 2-chloroethanol). The base radical cation $B^{\dot{+}}$ or other transients could not be observed since probably their lifetimes are short and/or their extinction coefficients at $\lambda > 300$ nm are too small. The number of absorbed photons was calculated from the incident laser energy using ϵ_{eff} (for energy measurements see Section 2.3). With KI in aqueous solution $\Phi_{e^-} = 0.36$ was obtained in agreement with a published value [20]. No effect of membrane filtration on Φ_{e^-} was found for the nucleic acids.

2.5. Quantum yield of single-strand break formation

The solutions (OD = 2.5 at λ_{max}) were repeatedly flashed (pulse frequency, 0.3 - 1 Hz) and stirred in the quartz cell ($l = 1$ cm; $V = 7.5$ ml) by purging with argon or N_2O. $\bar{M}_w$ values were determined by employing a low angle light-scattering apparatus (Chromatix KMX-6) [15] at scattering angles of 6° - 7°; prior to the light-scattering analysis the solutions were adjusted to a concentration of 0.5 M in KCl and 0.1 M in TRIS buffer (pH 8). The number n_{ssb} of single-strand breaks was calculated according to the equation

$$n_{\text{ssb}} = N_L V c_{\text{ss}} \left\{ \frac{\bar{M}_w(\text{initial})}{\bar{M}_w(\text{final})} - 1 \right\} \tag{2}$$

where N_L is Avogadro's number and c_{ss} the initial concentration of single strands; c_{ss} is calculated from the number-averaged molecular weight $\bar{M}_n$ which is $0.5\bar{M}_w$ for a random size distribution [21]. Φ_{ssb} was obtained from n_{ssb} and the number of absorbed photons was calculated from the pulse energy. Owing to the high concentrations, virtually all incident photons were absorbed. For monophotonic excitation the samples were irradiated with a low pressure mercury lamp ($\lambda = 254$ nm) equipped with a Vycor filter to suppress shorter wavelengths.

3. Results

3.1. Conductivity change

Upon laser excitation of poly A, poly C, poly U and ssDNA in argon-saturated aqueous solution a similar time dependence of the conductivity increase was detected for the four nucleic acids over the whole pH range studied, consisting of two parts: an initial increase (amplitude $\Delta\kappa_1$), which appears within the response time of our detection system (10 μs), and a much slower increase (amplitude $\Delta\kappa_2$) in the millisecond range, as recently reported for poly U [7]. An example of $\Delta\kappa$ as a function of time is shown in the inset of Fig. 1 for poly C. The conductivity increase is the result of a decrease in the fraction of bound counterions (which are condensed owing to the high charge density at the strand) with decreasing chain length of the polymer [13]. For poly U, poly C and ssDNA (but not for poly A) the slow increase could usually be fitted by a single-exponential function (rate constant, k_{obs}). In the case of ssDNA and poly A, additionally a third and very slow conductivity increase was observed at a pH of about 7 ($k''_{obs} \approx 0.3\ s^{-1}$)

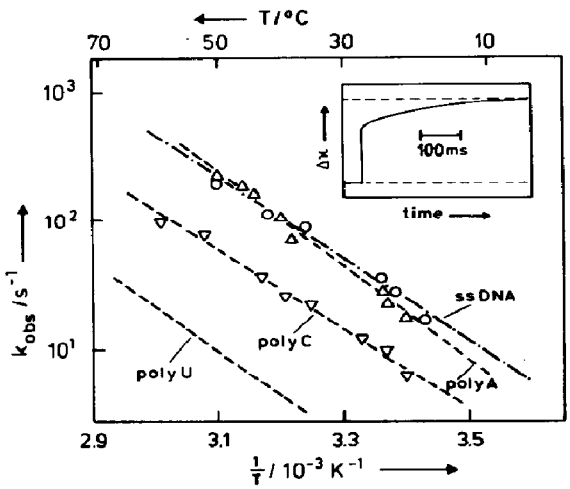


Fig. 1. Effect of temperature on the laser-induced conductivity increase: k_{obs} as a function of T^{-1} for poly A (△), poly C (▽) and ssDNA (○) at pH 7.3. The result for poly U (pH 6.8) (— — —) is taken from ref. 7. Inset: oscilloscope trace of a typical conductivity increase of poly C.

with a maximum amplitude of 30% of $\Delta\kappa_2$. The slow component of poly A solutions could not be described by a single-exponential function at a pH of 7 or below but in the semilogarithmic plots of ln $\Delta\kappa$ *vs.* time a good approximation could be achieved by applying two reaction rate constants: k_{obs}, which coincides very well with the inverse of the first half-life ($(\ln 2)\tau^{-1}$), and k'_{obs} (which is about $0.5k_{obs}$). At elevated temperatures the slow component could be described with one single-exponential function. The following results refer to the slow reaction rate constant k_{obs} for poly U, poly C and ssDNA and to $(\ln 2)\tau^{-1}$ in the case of poly A (Table 1).

TABLE 1

Arrhenius parameters and quenching rate constants with dithiothreitol for the laser-induced slow conductivity increase of nucleic acids[a]

	k_{obs} (s^{-1})	E_a (kJ mol^{-1})	A (s^{-1})	k_{DTT} (M^{-1} s^{-1})
poly U[b]	1.0 ± 0.1	66	5×10^{11}	1.2×10^6
poly C	8 ± 1	58	1.2×10^{12}	1.7×10^6
poly A	23 ± 2	69	5×10^{13}	$< 0.7 \times 10^6$
ssDNA	27 ± 2	57	3×10^{11}	$< 0.7 \times 10^6$

[a]At 23 °C and pH 7.3, except for poly U (pH 6.8).
[b]Taken from ref. 7.

3.1.1. Dependence on temperature

An increase in k_{obs} was found on increasing the temperature at pH 7.3 (Fig. 1). The activation energies E_a, calculated from the slope of the linear dependence of $\log k_{obs}$ *vs.* T^{-1}, and the pre-exponential factors A are presented in Table 1 together with the known values for poly U [7].

3.1.2. Dependence on pH

The dependence of k_{obs} on pH at 23 °C is shown in Fig. 2 for poly U [7], poly A, poly C and ssDNA. For poly C the rate constant decreases at a pH of 6.5 or below (results not shown). In contrast to poly U and poly C, k_{obs} increases with pH (at a pH above 7) for poly A and ssDNA.

3.1.3. Dependence on dithiothreitol concentration

For poly C, addition of DTT results in a significant increase in k_{obs} (Fig. 3) in conjunction with a decrease in the initial amplitude of the slow component. From the slope of k_{obs} *vs.* [DTT] a bimolecular rate constant $k_{DTT} = 1.7 \times 10^6$ M^{-1} s^{-1} was determined. The observed initial amplitude $\Delta\kappa_2$ as a function of the DTT concentration can be calculated using the equation

$$\Delta\kappa_2(\mathrm{DTT}) = \Delta\kappa_2(0)\frac{k^{\circ}_{obs}}{k^{\circ}_{obs} + k_{DTT}[\mathrm{DTT}]} \quad (3)$$

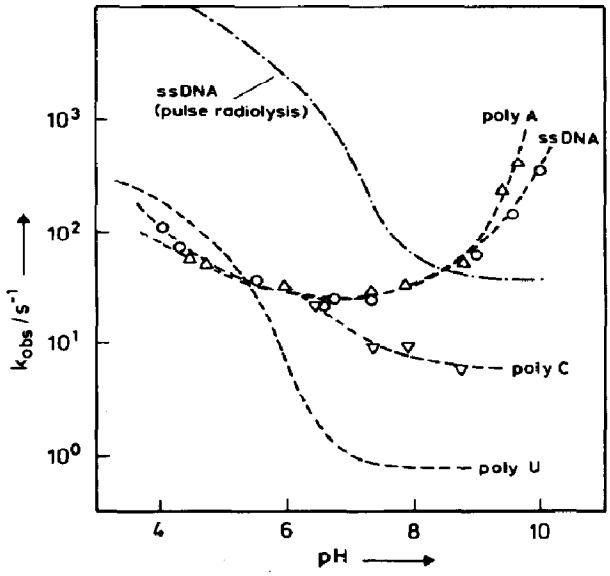


Fig. 2. Effect of pH on the laser-induced conductivity increase: pH dependence of k_{obs} for poly A (△), poly C (▽) and ssDNA (○) at 23 °C. The result for poly U (— — —), found for laser excitation and pulse radiolysis, is taken from ref. 7. For ssDNA (— · —) upon pulse radiolytic induction see ref. 22.

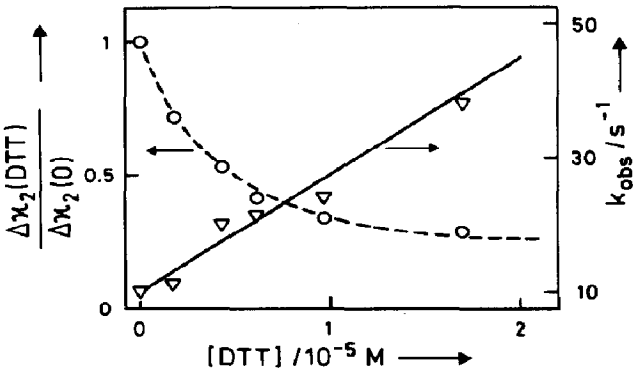


Fig. 3. Effect of the DTT concentration on the laser-induced slow conductivity increase: k_{obs} (▽) and $\Delta\kappa_2$ (○) as a function of [DTT] for poly C at pH 7.3 and 23 °C. — — —, initial amplitudes calculated from eqn. (3).

For poly A and ssDNA no significant influence of added DTT (up to 5×10^{-5} M) on the conductivity increase could be detected. An upper limit of $k_{DTT} = 7 \times 10^5$ M^{-1} s^{-1} can be given for the reaction of radicals of these nucleic acids with DTT.

3.2. Quantum yields

3.2.1. Effective absorption coefficient upon laser excitation

The dependence of the effective absorption coefficients ϵ_{eff}, upon laser excitation (λ_{exc} = 248 nm), on the incident intensity (I_L) is shown in Fig. 4. The values for $I_L \rightarrow 0$ are taken from the steady state UV spectrum under

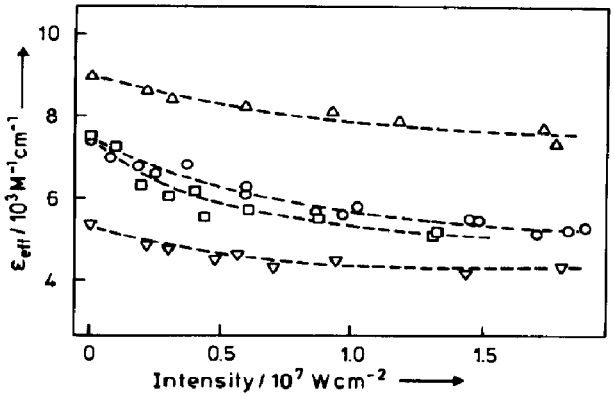


Fig. 4. Effective absorption coefficient at 248 nm as a function of the incident laser intensity at 23 °C for poly A (△), poly C (▽), ssDNA (○) at pH = 7.3, and poly U (□) at pH = 6.8.

monophotonic absorption conditions at 248 nm. ϵ_{eff} decreases slightly with I_L for the four nucleic acids, and this is probably caused by saturation effects in one of the biphotonic excitation steps.

3.2.2. Quantum yield of ionization and decay of the hydrated electrons

For the four nucleic acids the dependence of Φ_{e^-} on the incident laser intensity I_L is shown in Figs. 5 - 8. For poly U Φ_{e^-} increases with I_L, from

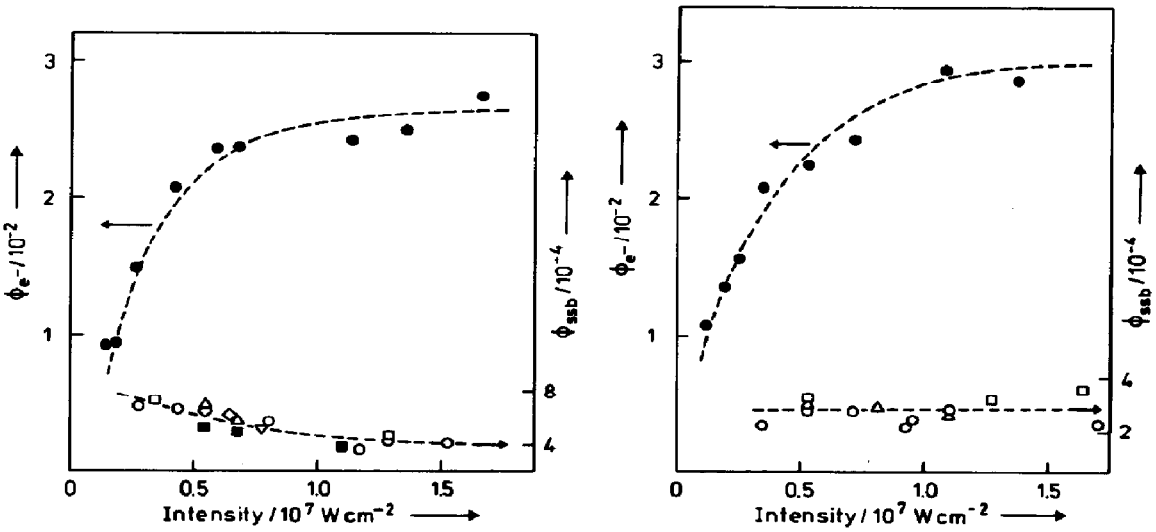
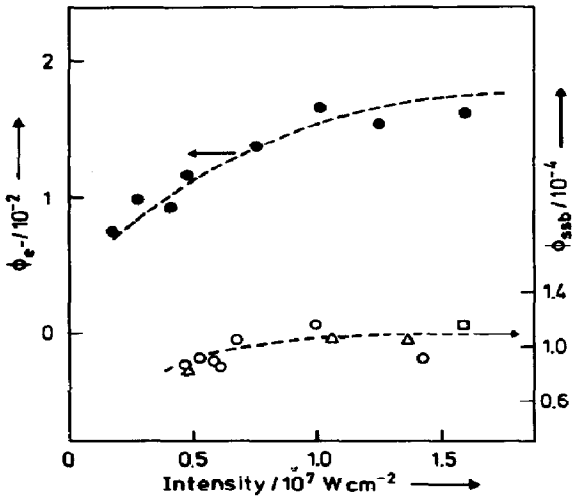


Fig. 5. Quantum yields of ionization, Φ_{e^-} (●), and ssb formation, Φ_{ssb}, for poly U as a function of the incident laser intensity at pH 6.8 and 23 °C. ○, Φ_{ssb} in argon-saturated solutions without additives. With additives: △, [DTT] = 1×10^{-5} M; ◇, [*t*-butanol] = 7×10^{-2} M; ▽, [$ClCH_2CH_2OH$] = 0.1 M. □, N_2O-saturated solutions without additives. ■, N_2O-saturated solution with [*t*-butanol] = 3×10^{-2} M.

Fig. 6. Quantum yields Φ_{e^-} (●) and Φ_{ssb} for poly C as a function of the incident laser intensity at pH 7.3 and 23 °C. ○, Φ_{ssb} in argon-saturated solutions without additives. △, with [DTT] = 2×10^{-5} M. □, in N_2O-saturated solutions.



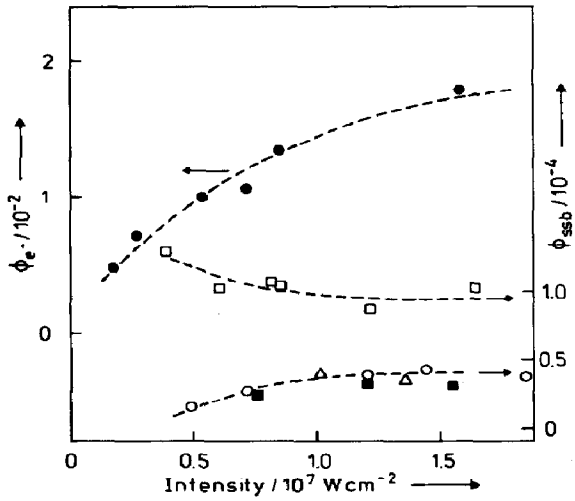


Fig. 7. Quantum yield Φ_{e^-} (●) and Φ_{ssb} for poly A as a function of the incident laser intensity at pH 7.3 and 23 °C. ○, Φ_{ssb} in argon-saturated solutions without additives. △, with [DTT] = 2×10^{-5} M. □, in N_2O-saturated solutions.

Fig. 8. Quantum yield Φ_{e^-} (●) and Φ_{ssb} for ssDNA as a function of the incident laser intensity at pH 7.3 and 23 °C. ○, Φ_{ssb} in argon-saturated solutions without additives. △, with [DTT] = 2×10^{-5} M. □, N_2O-saturated solutions without additives. ■, N_2O-saturated solutions with [*t*-butanol] = 3×10^{-2} M.

about 9×10^{-3} at $I_L < 1.6 \times 10^6$ W cm^{-2}, reaching saturation values $\Phi_{e^-}{}^{max}$ around 3×10^{-2} at $I_L > 1 \times 10^7$ W cm^{-2} (Table 2). A similar dependence was found for poly C, with a slightly lower $\Phi_{e^-}{}^{max}$ value for poly A and ssDNA.

The decay of the hydrated electrons produced upon ionization of poly U in argon-saturated solutions was found to be independent of the poly U concentration ((0.4 - 2.5) $\times 10^{-4}$ M) up to a laser intensity of 4×10^6 W cm^{-2}. The rate constant for the decay under these conditions was 3×10^5 s^{-1}. The reaction rate constant of the hydrated photoelectrons of poly U with 2-chloroethanol was found to be 7.0×10^8 M^{-1} s^{-1}, in close agreement with a literature value of 6.4×10^8 M^{-1} s^{-1} for electrons produced by pulse radiolysis [23]. Upon saturation with N_2O, the lifetime of the hydrated electrons was shorter than the laser pulse width (20 ns).

For the biphotonic excitation process it is assumed that upon nanosecond excitation the second photon is absorbed by the triplet state owing to the short lifetime of the S_1 state of the nucleic acid bases [3, 9]. Therefore the intersystem crossing quantum yield Φ_{isc} should be a limiting factor for the quantum yield of the biphotonic absorption process. In the case of poly U, $\Phi_{e^-}{}^{max} = 2.9 \times 10^{-2}$ is higher than the reported value Φ_{isc} = (1.4 - 2.3) $\times 10^{-2}$ for uracil [3, 10, 11]. Furthermore, this $\Phi_{e^-}{}^{max}$ value can be compared with results obtained for poly U on picosecond laser excitation at the same pulse energy (but at $I_L \approx 10^{10}$ W cm^{-2}), where the second photon is thought to be absorbed by the S_1 state. In this case Φ_{isc} should not be the limiting factor for the biphotonic excitation. However, the reported value $\Phi_{e^-}{}^{max} = 4.5 \times 10^{-2}$ [6] is only slightly higher than our value for nanosecond

TABLE 2

Quantum yields of ionization and single-strand break formation of nucleic acids[a]

	$\Phi_{e^-}^{max}$ ($\times 10^{-2}$)	Φ_{ssb} ($\times 10^{-4}$)	$\Phi_{e^-}^{max}/\Phi_{ssb}$
poly U	2.9	5.0	60
poly C	2.8	2.6	110
poly A	1.7	1.1	150
ssDNA	1.9	0.5	380

[a]At 23 °C and pH 7.3, except for poly U (pH 6.8).

excitation. For the DNA bases Φ_{isc} ranges from $(1 - 2) \times 10^{-3}$ for guanine and adenine [3] to 1×10^{-2} for thymine and cytosine [3, 10, 11], which coincides with Φ_{e^-} obtained upon nanosecond excitation of nucleosides: $\Phi_{e^-} = 2.4 \times 10^{-3}$ for adenosine, $\Phi_{e^-} = 2.8 \times 10^{-3}$ for guanosine and $\Phi_{e^-} = 1.7 \times 10^{-2}$ for thymidine ($I_L = (1.3 - 1.9) \times 10^7$ W cm^{-2}). These values are lower than that for ssDNA ($\Phi_{e^-}^{max} = 1.9 \times 10^{-2}$). This result raises some questions concerning the biphotonic excitation mechanism in the case of nucleic acids. Apart from the possibility that Φ_{isc} is different for nucleic acids, it is conceivable that a certain part of the biphotonic absorption process occurs via S_1–S_1 annihilation or that (retaining the idea that the T_1 state absorbs the second photon) the monophotonic energy is first transferred to the pyrimidines, where the intersystem crossing is more efficient.

3.2.3. Quantum yield of chromophore loss

For poly U the quantum yield Φ_{cl} of chromophore loss was found to be 0.024 ($I_L = 8 \times 10^6$ W cm^{-2}). Boiling the flashed solution (60 min, 90 °C, pH 1.5) led to 54% recovery of the chromophore loss; prolonged boiling (120 min) had no further effect.

3.2.4. Quantum yield of single-strand break formation

Φ_{ssb} as a function of the incident laser intensity is shown in Figs. 5 - 8. It must be stressed that Φ_{ssb} was determined under conditions of total absorption of the incident energy owing to the high optical densities required for light-scattering analysis (see Section 2). Thus the number of absorbed photons corresponds in part to intensities which are too low for a biphotonic excitation process. To estimate this contribution, Φ_{ssb} was analysed at different intensities covering both ends of the nearly constant part of Φ_{e^-} *vs.* I_L ($(0.5 - 1.5) \times 10^7$ W cm^{-2}). Φ_{e^-} is assumed to be equal to the quantum yield of the radical cations, which are supposed to induce ssb formation. Since within experimental error Φ_{ssb} is constant, a significant influence on Φ_{ssb} is expected only for $I_L < 0.5 \times 10^7$ W cm^{-2}, based on the behaviour of Φ_{e^-} *vs.* I_L. Thus the error introduced into the calculation of Φ_{ssb} (resulting from the overall determination of the number of photons) is estimated to be less than 20%. No significant change in Φ_{ssb} was found for poly U on addition of DTT (1×10^{-5} M), *t*-butanol (7×10^{-2} M) or 2-chloroethanol (0.1 M), upon saturation with N_2O or upon addition of *t*-butanol

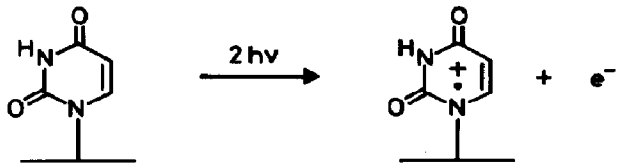
(3×10^{-2} M) to N_2O-saturated solutions. Under monophotonic excitation conditions ($\lambda = 254$ nm), Φ_{ssb} values were found to be about 1% of those obtained for laser excitation.

4. Discussion

4.1. Poly U

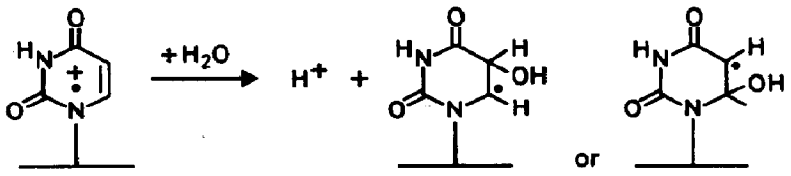
4.1.1. Mechanism of single-strand break formation of polyuridylic acid

In our recent study on the laser-induced ssb formation of poly U in argon-saturated aqueous solution [7] it was shown that the kinetic parameters for the slow conductivity increase are very similar to those obtained under OH-radical-induced conditions [13]. We therefore concluded that the rate-determining step is identical for the two kinds of initiation of ssb formation. For poly U, we suggested that the first step in the mechanism for ssb formation is the biphotonic ionization of the base moiety, which produces a hydrated electron and a radical cation of the base ($B^{+\cdot}$).



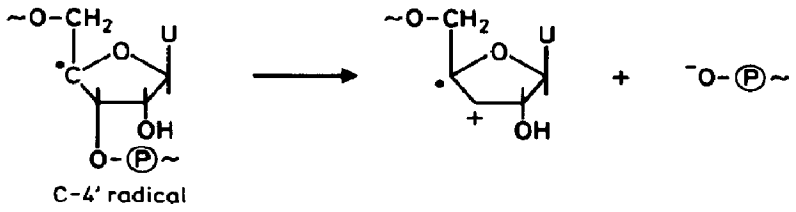
(4)

Since the hydrated electron does not contribute to ssb formation [15], the fate of $B^{+\cdot}$ has to be considered. There is some evidence that one reaction of $B^{+\cdot}$ is water addition, forming $OH^{\cdot}$ addition products at position 5 or 6 [24].

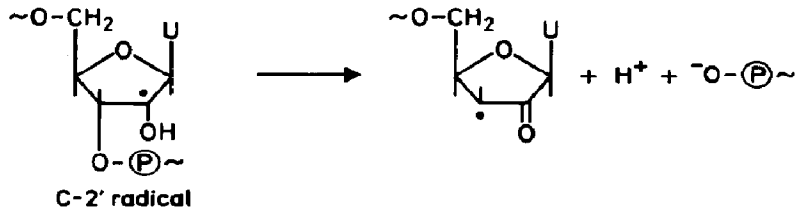


(5)

The uracil radical abstracts a hydrogen atom from the sugar moiety. Sugar radicals at C(2)′ or C(4)′ lead to ssb formation by heterolytic elimination of the phosphate ester bond.



(6a)



(6b)

With pulse radiolysis it has been found that 70% of the OH radicals add at the C(5) position of the C(5)–C(6) double bond of the uracil moiety (forming a 6-yl radical), 23% add at the C(6) position (forming a 5-yl radical) and 7% abstract a hydrogen atom from the sugar [25]. Since the efficiency of ssb formation by OH radicals in anoxic solutions is 41% [15] it was concluded that it is mainly the C(5)–OH adduct (the 6-yl radical) which causes ssb formation.

Therefore, from the identity of the rate-determining step for the biphotonic and the OH-radical-induced processes it can be assumed that one photolytically produced precursor of ssb formation is the C(5)–OH adduct which leads to ssb formation by hydrogen-atom abstraction from the sugar moiety. Recent pulse radiolytic results for poly U have shown that the rate constant for the conductivity increase has a pH dependence very similar to the rate constant for the disappearance of intermediate base radicals [14], which is evidence that the rate-determining step is the hydrogen-atom abstraction from the sugar by intermediate base radicals.

4.1.2. Possible explanations for the difference between Φ_{ssb} and $\Phi_{B^{\ddot{+}}}$

A comparison between $\Phi_{B^{\ddot{+}}}$ and Φ_{ssb} shows that Φ_{ssb} is only about 2% of $\Phi_{B^{\ddot{+}}}$ if the latter yield can be assumed to be equal to Φ_{e^-}. Upon γ radiolysis, where the strand break formation is dominated by OH radicals, 41% of the OH radicals lead to ssb formation in deoxygenated solution [15]. If it is assumed that every $B^{\ddot{+}}$ reacts with water and is converted into a uracil radical (5-yl or 6-yl) and that the ratio of the 5-yl to the 6-yl radicals is similar to that generated by OH radicals, then Φ_{ssb} should be $0.41\Phi_{B^{\ddot{+}}} = 1.2 \times 10^{-2}$. The actual value ($5 \times 10^{-4}$) is only 4% of the expected value. Two conceivable reasons for this discrepancy will be discussed: (a) the influence of a different 5-yl/6-yl radical ratio and (b) back reactions of an ion pair or a radical pair.

(a) If it is assumed that the 5-yl radical does not (or only to a minor extent) lead to ssb formation, the dominant production of this radical, as a product of the reaction of $B^{\ddot{+}}$ with water, would explain the low ratio of Φ_{ssb} to Φ_{e^-}. An estimation of the 5-yl radical yield can be made if it is assumed that this radical is converted into the C(6) hydrate by $H^{\cdot}$ abstraction from the sugar leading to a chromophore loss at 260 nm but not to ssb formation. The yield of the C(6) hydrate upon laser excitation was checked by determining the degree of reversibility of this chromophore loss. From the quantum yield $\Phi_{cl} = 2.4 \times 10^{-2}$ and 54% reversibility a quantum yield Φ_H for the C(6) hydrate formation of 1.2×10^{-2} was calculated. This, how-

ever, is too low to account for the apparent difference between Φ_{ssb} and $\Phi_{B^{\cdot}}$. In addition, an unknown amount of C(6) hydrate may be formed photochemically by a one-quantum process leading to addition of water directly to the electronically excited state of the uracil base [26]. Consequently, Φ_H also contains contributions from the monophotonically produced photohydrate in addition to that formed by $H^{\cdot}$ abstraction from the sugar by the 5-yl radical. Schuchmann *et al.* gave a further argument against a high 5-yl to 6-yl radical ratio in ref. 27; they found that the product of the reaction of 1,3-dimethyluracil with the oxidizing $SO_4^{\cdot-}$ radical (probably a radical cation) is preferentially converted into the 5-OH,6-yl radical on reaction with water.

(b) Apart from the low Φ_{ssb}/Φ_{e^-} ratio we found two further discrepancies between the results of laser photolysis and γ radiolysis. When the radiolytically produced hydrated electron is scavenged by N_2O an OH radical is formed according to

$$e^-_{aq} + N_2O + H_2O \longrightarrow OH^{\cdot} + OH^- + N_2 \qquad (7)$$

OH radicals formed radiolytically in this way react with poly U and give rise to ssb formation with 41% efficiency [15]. When electrons are produced by photoionization a marked reduction in the lifetime in the presence of N_2O indicates that there is also a conversion into OH radicals according to eqn. (7). However, in the presence of N_2O, Φ_{ssb} is not enhanced and no difference could be detected between the behaviour of argon- and N_2O-saturated solutions (Fig. 5). Thus Φ_{ssb} is smaller by a factor of more than 20 than would be expected on the basis of a comparison with γ radiolysis.

In the case of γ radiolysis, the addition of N_2O and *t*-butanol results in a decrease in ssb formation owing to effective scavenging of OH radicals [15]. Upon laser excitation no reduction in ssb formation was observed on addition of N_2O and *t*-butanol (Fig. 5), even though if the OH radicals were homogeneously distributed 97% of them should be scavenged, taking k(OH + poly U) = 1.5×10^9 M^{-1} s^{-1} [13], k(OH + *t*-butanol) = 5.2×10^8 M^{-1} s^{-1} [28], [*t*-butanol] = 3×10^{-2} M and [poly U] = 3×10^{-4} M.

We give the following explanation for these discrepancies. It could be that the distance between the hydrated electron and the radical cation of the base, when produced by photoionization, is so small that the electron and the radical cation may form some kind of complex or a geminate pair of radical ions. Such intermediates have already been postulated by several groups, *e.g.* by Grossweiner and coworkers for tryptophan in water [29, 30], by Ebbesen and Previtali for phenol in alcohols [31], by Hirata and Mataga for tetramethylphenylendiamine in alcohols [32] or by Nakamura *et al.* in acetonitrile [33]. A complex or a geminate pair of radicals could lead to a fast geminate recombination or to a fast geminate combination of possibly transformed but still correlated radicals of a pair.

The results that the conversion of the photoelectrons into OH radicals by N_2O does not lead to an increase in Φ_{ssb} and that addition of *t*-butanol does not affect Φ_{ssb} (although significant effects are expected from the com-

parison with γ radiolysis results) could be explained by assuming that the OH radicals produced from photoelectrons are still correlated to the radical cations and that fast combination can occur. We assume that this correlation is also maintained after conversion of the radical cation into another radical, *e.g.* after the reaction with water (eqn. (5)), prior to combination with the counter-radical. This reaction could be so fast that scavenging by *t*-butanol may not compete.

It is expected that a glycol derivative will be produced in the reaction of OH radicals with radicals derived from the reaction of $B^{+\cdot}$ with water. A similar pathway is assumed for the reaction of poly U with the scavenging product of the hydrated electron with 2-chloroethanol

$$e^-_{aq} + ClCH_2CH_2OH \longrightarrow Cl^- + {}^{\cdot}CH_2CH_2OH \qquad (8)$$

The resulting radical could react with the correlated radical cation or its products.

Our interpretation in terms of geminate combination is supported by comparing the results for the lifetime of the hydrated electrons produced either by photoionization or by pulse radiolysis. In the former case the lifetime of the hydrated electrons in argon-saturated aqueous poly U solution (concentration, 1×10^{-4} M at pH 6.8) is shorter than or equal to 2 μs, *i.e.* it is much shorter than under pulse radiolysis conditions (longer than 20 μs), taking $k(e^- + \text{poly U}) = 3 \times 10^8\ M^{-1}\ s^{-1}$ [34]. The linear increase in the decay rate constant with the poly U concentration is in contrast to the results for laser excitation, where the decay was found to be independent of the poly U concentration ($(0.4 - 2.5) \times 10^{-4}$ M) up to a laser intensity of 4×10^6 W cm^{-2}. This discrepancy may be explained in terms of fast geminate combination of the hydrated electron with $B^{+\cdot}$ or with a radical produced after reaction of $B^{+\cdot}$, *e.g.* with water. In contrast, the radiolytically produced hydrated electrons in aqueous solution are homogeneously distributed a few nanoseconds after the electron pulse and they decay randomly by reaction with poly U.

This combination mechanism could be an explanation for the low $\Phi_{ssb}/\Phi_{B^{+\cdot}}$ ratio, if the hydrogen-atom abstraction from the sugar is much slower than the geminate combination. This is the case at least for the slow component of ssb formation, for which hydrogen-atom abstraction is interpreted to be the rate-determining step (see Section 4.1.1. and ref. 14).

In summary, it can be said that there are four arguments in favour of a fast and effective combination of a geminate pair of radicals and/or radical ions produced by photoionization. (a) The difference between $\Phi_{B^{+\cdot}}$ and Φ_{ssb} of a factor of about 60. (b) The lack of an increase in Φ_{ssb} on addition of N_2O. Since electrons are converted into OH radicals in the presence of N_2O an increase by a factor of more than 20 is expected on the basis of results from γ radiolysis. (c) The absence of an influence of *t*-butanol on Φ_{ssb} under conditions where all hydrated electrons are converted into OH radicals. (d) The much smaller lifetime of the hydrated electrons, if produced by laser excitation of poly U, in comparison with that obtained by pulse radiolysis, and the different concentration dependence in the two cases.

4.1.3. Effect of dithiothreitol on single-strand break formation

Investigation of ssb formation of poly U by light scattering and conductivity measurements shows that Φ_{ssb} is not affected within experimental error by a DTT concentration of 1×10^{-5} M, whereas the slow conductivity increase is quenched with a rate constant $k_{DTT} = 1.2 \times 10^{6} M^{-1} s^{-1}$ [7]. At this concentration the slow conductivity increase is reduced to less than 10% of its initial value. Since the slow component governs about 20% of the total conductivity increase this reduction upon DTT addition is only within the experimental error of the Φ_{ssb} determination by light scattering if it is assumed that both the fast and the slow conductivity increases reflect ssb formation. If only the slow component is correlated to ssb formation a reduction of Φ_{ssb} by 90% is expected which would be detected by molecular weight determination.

4.1.4. Estimation of the contribution of radicals from water decomposition in single-strand break formation

In addition to the reaction of the radical cations [7], another reaction pathway was suggested by Nikogosyan *et al.* [6] which involves an excitation energy transfer from the excited base to the aqueous solvent thereby forming electronically excited water molecules with an excitation energy exceeding the limit for ionization (6.5 eV) and dissociation (5 eV). This process was assumed for thymine [35] and uridine-5′-monophosphate [36] on the basis of a reduced extent of photobleaching on addition of *t*-butanol as a scavenger of the postulated OH radicals. In the case of polynucleotides, it is proposed that ssb formation is induced by OH and H radicals as products of this excitation. If these radicals are homogeneously distributed, efficiencies for ssb formation of 41% and 19% are expected for the reaction of poly U with OH and H radicals respectively [15]. In this case the addition of *t*-butanol, which acts as an efficient scavenger of OH radicals, should reduce the OH-radical-induced ssb formation by 95%, using the above-mentioned rate constants for the reaction of OH radicals with poly U or *t*-butanol. As shown in Fig. 5, Φ_{ssb} is not significantly reduced and therefore a substantial participation of homogeneously distributed OH radicals in ssb formation can be excluded under our excitation conditions. The same arguments exclude the significant participation of OH and H radicals possibly produced by direct biphotonic excitation of the solvent water upon excitation with a nanosecond laser pulse. Such a direct photolysis of water has been reported using picosecond laser excitation at higher intensities [37].

4.2. Polycytidylic acid

For poly C the conductivity increase, especially the pH dependence of k_{obs} at a pH of above 6.5, resembles the behaviour of poly U (Fig. 2). The decrease in k_{obs} for a pH of below 6.5 can be explained by the fact that poly C becomes double stranded below this pH [38, 39]. The slow component is quenched by DTT with a rate constant (1.7×10^{6} M^{-1} s^{-1}, Fig. 3) compa-

rable with that for poly U (1.2×10^6 M^{-1} s^{-1} [7]). It may therefore be concluded for poly C that the mechanism of ssb formation is analogous to that for poly U. Comparing the intensity dependence of Φ_{e^-} and Φ_{ssb} for poly U (Fig. 5) and poly C (Fig. 6) a decrease in Φ_{ssb} with decreasing intensity, which should be expected from the behaviour of Φ_{e^-} *vs.* I_L, was not observed. This needs further investigation. Monophotonic processes lead to ssb formation with much lower efficiencies (see Section 3) but it is possible that other processes, *e.g.* cross-linking at higher intensities, account for this behaviour.

4.3. Polyadenylic acid and single-stranded DNA

The pH dependence of k_{obs} is very similar for poly A and ssDNA, but different from that of poly U and poly C (Fig. 2). For poly U and poly C k_{obs} decreases with pH whereas for poly A and ssDNA k_{obs} has a minimum at a pH of about 7 and increases again above pH 7. Furthermore, addition of DTT (up to 5×10^{-5} M) has no significant effect on the conductivity increase for poly A and ssDNA in contrast to poly U [7] and poly C (Fig. 3). Since in all four cases radical transfer from the base radicals to the sugar is assumed as a pathway to ssb formation, and since for poly U hydrogen-atom abstraction is now proposed to be the rate-determining step [14], we also assume that for poly A and ssDNA hydrogen atom abstraction by base radicals is the rate-determining step. The differences in the rate constant, the pH dependence and the effect of DTT for the four nucleic acids are then explained by the differences in character of the radicals involved in the rate-determining step for the slow ssb formation of the "pyrimidine" polynucleotides on the one hand and of poly A and ssDNA on the other.

The similarity of the pH effect on k_{obs} for poly A to that for ssDNA (Fig. 2) suggests that the slow ssb formation in ssDNA is related to purine bases. This may indicate charge or excitation energy transfer along the chain in ssDNA, leading to preferential ionization of purine moieties in the case of biphotonic excitation. It was not possible to determine which of the purine bases, adenine or guanine, is ionized, because the corresponding model polynucleotide, poly G, is multiple stranded and not available in the single-stranded form [38, 39]. However, the formation of a guanine radical cation as an intermediate is possible since poly G is cleaved by photosensitization using tris(2,2′-bipyridyl)ruthenium(II) and $S_2O_8^{2-}$. For this system it has been postulated that ssb formation is initiated by a one-electron oxidation of the guanine moiety [40]. A distinction between a "pyrimidine" and a "purine" mechanism of ssb formation based on the activation energies for the slow conductivity increase was not possible, since the obtained values are similar for the four nucleic acids (Table 1).

The finding that charge or excitation energy transfer along the ssDNA chain may occur is of great interest concerning the theory of Adams who postulated migration of charges along the strand in order to explain some biological effects of high energy irradiation [41, 42]. An excitation energy transfer along the DNA chain in UV-irradiated aqueous glasses at 77 K was

found by Gräslund *et al.* [43]. The main products detected were thymine radical anions and guanine radical cations. Boon *et al.* have provided additional information by showing that γ irradiation of a frozen aqueous DNA solution at 77 K, where only guanine radical cations and thymine radical anions could be detected, results in dsb and ssb formation after melting the sample. The authors concluded that one or both of these radicals are involved in the strand break formation of DNA [44].

In contrast, radiolytically produced OH radicals react with the four bases of DNA approximately with equal rate constants [45]. Therefore, the kinetics of ssb formation of ssDNA, induced by OH radicals, should be initiated by pyrimidine and purine base radicals. Since the pH dependence of k_{obs} is similar for ssDNA and poly U (Fig. 2) it may be assumed that ssb formation in ssDNA, induced by pyrimidine base radicals, has a higher yield than that induced by purine base radicals. In agreement, the yield of ssb formation is higher for poly U than for poly A [15, 46].

Figure 8 shows that for ssDNA Φ_{ssb} is slightly greater in the presence of N_2O (where the electrons are converted into OH radicals) compared with in argon-saturated solutions. This indicates that a small percentage of the photoelectrons lose the spatial correlation with their counter-radical cations (or products of these) and, upon conversion into OH radicals, a reaction outside the cage is possible. The decrease in Φ_{ssb} upon addition of *t*-butanol to N_2O-saturated solutions (Fig. 8) is consequently attributed to a scavenging effect of these uncorrelated OH radicals by *t*-butanol. For γ radiolysis of ssDNA it has been found that 15% of the OH radicals induce ssb formation [17]. Therefore upon laser excitation Φ_{ssb} is smaller by a factor of 30 than the value expected if all B^{+} reacts with water and no back reaction occurs. This supports our assumption that a geminate pair of radicals and/or radical ions is formed which decays by a fast combination as the main reaction channel.

5. Conclusions

The presented results show that radical cations of the bases lead to ssb formation in the case of poly A, poly C, poly U and ssDNA. The low yield for ssb formation, obtained by laser-induced ionization, is not necessarily caused by low reactivity of the radical cations produced on the strand since evidently the main reaction channel is a fast combination of reactive intermediates. It may be that radical cations produced by the direct effect (direct ionization of DNA by high energy irradiation) lead to ssb formation with a higher yield than with laser excitation.

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References

1 P. G. Kryukov, V. S. Letokhov, D. N. Nikogosyan, A. V. Borodavkin, E. I. Budowsky and N. A. Simukova, *Chem. Phys. Lett.*, *61* (1979) 375.
2 G. G. Gurzadyan, D. N. Nikogosyan, T. S. Balmukhanov and G. B. Zavilgelskij, *Photobiochem. Photobiophys.*, *4* (1982) 87.
3 D. N. Nikogosyan, D. A. Angelov and A. A. Oraevsky, *Photochem. Photobiol.*, *35* (1982) 627.
4 D. N. Nikogosyan and V. S. Letokhov, *Riv. Nuovo Cimento, Ser. 3*, *6* (8) (1983) 1.
5 D. N. Nikogosyan and G. G. Gurzadyan, *Laser Chem.*, *4* (1984) 297.
6 D. N. Nikogosyan, A. A. Oraevsky, V. S. Letokhov, Z. K. Arbieva and E. N. Dobrov, *Chem. Phys.*, *97* (1985) 31.
7 D. Schulte-Frohlinde, J. Opitz, H. Görner and E. Bothe, *Int. J. Radiat. Biol.*, *48* (1985) 397.
8 M. Johnson-Thompson, J. B. Halpern, W. M. Jackson and J. George, *Photochem. Photobiol.*, *39* (1984) 17.
9 M. Daniels and W. Hauswirth, *Science*, *171* (1971) 675.
S. Kobayashi, M. Yamashita, T. Sato and S. Muramatsu, *IEEE J. Quantum Electron.*, *20* (1984) 1383.
10 C. Salet and R. Bensasson, *Photochem. Photobiol.*, *22* (1975) 231.
11 G. J. Fisher, A. J. Varghese and H. E. Johns, *Photochem. Photobiol.*, *20* (1974) 109.
12 R. V. Bensasson, E. J. Land and T. G. Truscott, *Flash Photolysis and Pulse Radiolysis*, Pergamon, Oxford, 1983.
13 E. Bothe and D. Schulte-Frohlinde, *Z. Naturforsch., Teil C*, *37* (1982) 1191.
14 D. G. E. Lemaire, E. Bothe and D. Schulte-Frohlinde, *Int. J. Radiat. Biol.*, *51* (1987) 319.
E. Bothe and D. Schulte-Frohlinde, to be published.
15 D. G. E. Lemaire, E. Bothe and D. Schulte-Frohlinde, *Int. J. Radiat. Biol.*, *45* (1984) 351.
16 M. Müller, *Ph.D. Thesis*, University Bochum, F.R.G., 1983.
17 E. Bothe and M. Liphard, to be published.
18 R. Schrank, *Ph.D. Thesis*, University Bochum, F.R.G., 1980.
19 E. J. Hart (ed.), *Solvated Electron*, American Chemical Society, Washington, 1965.
E. J. Hart and M. Anbar (eds.), *The Hydrated Electron*, Wiley, New York, 1970.
20 P. Fornier de Violet, *Rev. Chem. Intermed.*, *4* (1981) 121.
21 A. Charlesby, *Proc. R. Soc. London, Ser. A*, *224* (1954) 120.
22 E. Bothe, G. A. Qureshi and D. Schulte-Frohlinde, *Z. Naturforsch., Teil C*, *38* (1983) 1030.
23 R. R. Hentz, Farhataziz and E. M. Hansen, *J. Chem. Phys.*, *57* (1972) 2959.
24 G. Behrens, J. Herak, K. Hildenbrand and D. Schulte-Frohlinde, to be published.
25 D. J. Deeble, D. Schulz and C. von Sonntag, *Int. J. Radiat. Biol.*, *49* (1986) 915.
26 G. J. Fisher and H. E. Johns, in S. Y. Wang (ed.), *Photochemistry and Photobiology of Nucleic Acids*, Academic Press, New York, 1976, p. 169.
27 H. P. Schuchmann, D. J. Deeble, G. Olbrich and C. von Sonntag, *Int. J. Radiat. Biol.*, in the press.
28 R. L. Willson, C. L. Greenstock, G. E. Adams, R. Wageman and L. M. Dorfman, *Int. J. Radiat. Phys. Chem.*, *3* (1971) 211.
29 F. D. Bryant, R. Santus and L. I. Grossweiner, *J. Phys. Chem.*, *79* (1975) 2711.
30 J. Y. Lee, J. F. Baugher and L. I. Grossweiner, *Photochem. Photobiol.*, *29* (1979) 867.
31 T. W. Ebbesen and C. M. Previtali, *J. Photochem.*, *31* (1985) 193.
32 Y. Hirata and N. Mataga, *J. Phys. Chem.*, *89* (1985) 4031.
33 S. Nakamura, N. Kanamaru, S. Nohara, H. Nakamura, Y. Saito, J. Tanaka, M. Sumitani, N. Nakashima and K. Yoshihara, *Bull. Chem. Soc. Jpn.*, *57* (1984) 145.
34 P. C. Shragge, H. B. Michaels and J. W. Hunt, *Radiat. Res.*, *47* (1971) 598.
35 A. A. Oraevsky and D. N. Nikogosyan, *Chem. Phys.*, *100* (1985) 429.

36 A. A. Oraevsky, D. N. Nikogosyan and V. S. Letokhov, *Lasers Life Sci.*, *1* (1986) 49.
37 D. N. Nikogosyan, A. A. Oraevsky and V. I. Rupasov, *Chem. Phys.*, *77* (1983) 131.
38 R. Steiner and D. B. S. Millar, in A. Veis (ed.), *Biological Polyelectrolytes*, Dekker, New York, 1970, p. 65.
39 P. O. P. Ts'O (ed.), *Basic Principles in Nucleic Acid Chemistry*, Academic Press, New York, 1974.
40 C. Stradowski and D. Schulte-Frohlinde, to be published.
41 G. E. Adams, in G. Stein (ed.), *Radiation Chemistry of Aqueous Systems*, The Weizmann Science Press, Jerusalem, 1968, p. 241.
42 G. E. Adams, in H. Moroson and M. Quintiliani (eds.), *Radiation Protection and Sensitization*, Taylor and Francis, London, 1970, p. 3.
43 A. Gräslund, A. Ehrenberg, A. Rupprecht and G. Ström, *Photochem. Photobiol.*, *29* (1979) 245.
44 P. J. Boon, P. M. Cullis, M. C. R. Symons and B. W. Wren, *J. Chem. Soc., Perkin Trans. II*, (1984) 1393.
45 *NSRDS–NBS 46*, 1973.
46 A. Önal, D. G. E. Lemaire, E. Bothe and D. Schulte-Frohlinde, to be published.