Rate of OH Radical Induced Strand Break Formation in Single Stranded DNA under Anoxic Conditions. An Investigation in Aqueous Solutions Using Conductivity Methods

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Single stranded DNA (ssDNA) in aqueous N_2O-saturated solution was pulse-irradiated with electrons or irradiated under steady-state conditions with ^{60}Co-γ rays. The conductivity increase after irradiation was measured as a function of concentration, pH, temperature, metal cation content and additives.

The conductivity increase could be shown to be due to the release of associated counterions (Na^+ or H^+) as a result of the formation of chain breaks. At 28 mg l^{-1} DNA $\sim$ 8 sodium ions are liberated per 100 eV absorbed energy ($G(Na^+) = 8.3$). On the basis of G value for single-strand breaks (ssb) of $G(\text{ssb}) = 0.55$, it is calculated that $8.3/0.55 = 15$ sodium ions per strand break are set free. The release of Na^+ monitored by ^{23}Na-NMR at pH 7 as a function of dose corresponds to that of the conductivity increase.

The rate of the conductivity increase does not depend on dose/pulse (range 2–20 J kg^{-1}) and shows at least two components. The rate constant of the fast and dominant component is constant above pH 8.5 ($k = 38\ s^{-1}$, 20 °C) and increases linearly with proton concentration below pH 7. Values of 13 kcal/mol for the activation energy and $5 \times 10^{11}\ s^{-1}$ for the frequency factor were obtained at pH 7.3. Addition of *p*-benzoquinone (pBQ) increases the rate constant of the fast component proportionally to the concentration of pBQ. From these results the rate-determining step of the fast conductivity increase is concluded to be the splitting of DNA radicals. The pH dependence and the magnitude of the activation energy agree with results from low molecular weight model compounds for the C-4′ mechanism; this mechanism involves a heterolytic splitting of the phosphoric acid ester bond starting from the 4′ radical of DNA.

Introduction

It is well documented [1, 2], that the reaction of radiation produced hydroxyl-radicals with DNA in aqueous solution leads to the formation of breaks in the sugar-phosphate backbone. Reaction mechanisms for strand break formation have been reviewed recently [3–5]. There is evidence, that the cleavage of the main chain originate in part from the 4′ radical of the deoxyribose (C-4′-mechanism) [6, 7]. The 4′ radical of the sugar may be produced directly from the OH radical by H abstraction or indirectly via a base radical. Indeed a recent study provides support for a contribution of the latter mechanism in case of poly U [8].

In a previous paper [9] we have demonstrated in the case of polyuridylic acid (poly U) that electric conductivity measurements are a sensitive and convenient method for the investigation of the rate of strand break formation in polyelectrolytes. Salts of polyelectrolytes exhibit a reduced conductivity in aqueous solutions as compared to the corresponding monomeric compounds. In terms of the two site model advanced by Manning [10–12] for an infinitly long polyanion, a certain fraction θ of the counterions is electrostatically bound to the polymeric chain, the remaining fraction $\alpha = 1 - \theta$ is free and subjected only to screening forces of the polymer.

As was found by Schindewolf [13] for polyphosphates, α increases with decreasing chain length. The introduction of chain breaks in a polyanion therefore increases the concentration of free counterions and hence also the conductivity of the solution.

The present paper deals mainly with the conductivity increase observed with solutions containing denatured DNA of low molecular weight after reaction with OH radicals. The OH radicals are

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produced in the aqueous N_2O-saturated solutions by ^{60}Co-γ and electron irradiation. The N_2O converts the radiation produced solvated electrons into OH radicals. The composition of the radicals reacting with DNA is 90% OH radicals and 10% H atoms. Since H atoms react with DNA in a similar way as OH radicals the contribution of the H atoms is not discussed separately.

From the results the rate constant of strand break formation in single stranded DNA (ssDNA) after reaction with OH radicals is obtained. It is shown that the rate of the conductivity increase reflects the rate of a chemical reaction leading to strand breaks. Furthermore some proposals are made concerning the chemical pathway leading to strand breaks.

Low molecular weight DNA of $\sim 5 \times 10^5$ g mol^{-1} was used as substrate because at molecular weights of $\geqq 1 \times 10^6$ g mol^{-1} a conductivity decrease was observed which will not be discussed in this paper.

Experimental

The experiments were carried out using the sodium salt of calf thymus DNA obtained from Merck, Darmstadt. The protein content was determined with the method of Lowry to be 2–4% and only $\leqq 0.3\%$ after treatment with Trypsin. However in both cases the same results were obtained in our conductivity experiments. Addition of albumin up to 15% of the DNA weight did not change significantly our results in pulse radiolysis. With albumin alone no conductivity change was found in pulse radiolysis. DNA was dissolved in six times distilled water at a concentration of 140 mg l^{-1}. Unless otherwise stated, the solutions were concentrated after 24 h by a factor of 10 in a membrane filtration apparatus (amicon, filter UM 10) and then rediluted with distilled water to the desired volume. Since the conductivity of the DNA solution is reduced as a result of that treatment it may be concluded that a certain number of small ions are removed. Oxygen was then removed by passing N_2O (oxygen content below 10 ppm) through the solutions for 45 min. After these procedures the pH of the solutions was measured to be 7.3 ± 0.3. Higher and lower pH values were adjusted by adding NaOH or $HClO_4$. The concentration of the solutions finally obtained was calculated from the UV absorbance at 258 nm and at 90 °C on the basis of an average molar extinction coefficient per nucleotide of 8500 l mol^{-1} cm^{-1}.

The described treatment by membrane filtration changed the hyperchromicity of the DNA. If untreated DNA was dissolved in distilled water at 0 °C the hyperchromicity was found to be $\sim 32\%$ and to occur in the range $30\,°C \leqq T \leqq 55\,°C$ (Fig. 1, squares). This degree of hyperchromicity is similar to that found in the literature at higher ionic strength for native DNA [14]. The hyperchromicity of the membrane filtered solutions was found to be around 8% in the temperature range 15 °C–95 °C at 140 mg l^{-1} DNA and approximately 7% at 35 mg l^{-1}. Thermal pre-treatment by heating the solution to 90 °C did not change the magnitude of this hyperchromicity by more than 2%. However, as can be seen from Fig. 1, the shape of the melting curve is quite different after heat treatment. Without heat treatment, there is a steep increase of the absorbance in the temperature range $30 \leqq T \leqq 50\,°C$ (Fig. 1, crosses), which provides evidence that DNA in aqueous solution without added salt still contains double stranded regions with a melting point of approximately 35 °C. After thermal denaturation no steep increase was observed, instead the absorbance increased continuously with increasing temperature in the range $15\,°C \leqq T \leqq 90\,°C$ (Fig. 1, circles). The

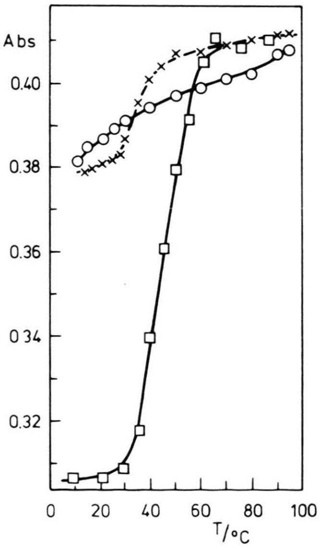

Fig. 1. Dependence of the absorbance of aqueous DNA solutions of 85 mg l^{-1} (observed in 0.2 cm cells) on temperature at pH = 7.3. □□ = DNA dissolved in distilled water at 0 °C; ×× = DNA dissolved in distilled water at room temperature and membrane-filtered; ○○ = after thermal treatment (5 min at 90 °C) following membrane filtration.

steep increase did not reappear even after allowing the solution to stand at room temperature for 24 h.

The average molecular weights $\bar{M}_w$ of DNA were determined by a low-angle laser light-scattering photometer (Chromatix KMX-6) in aqueous solutions containing 0.5 M NaCl (0.17 ml g^{-1} was adopted for d*n*/d*c*). Typical results were $\bar{M}_w = (5 \pm 2) \times 10^6$ g mol^{-1} for a variety of samples before sonication. Solutions of lower molecular weight DNA were obtained by sonicating the solutions of high molecular weight DNA. Ten minutes sonification resulted in $\bar{M}_w \approx 4 \times 10^5$ g mol^{-1}.

Conductivity changes were measured with DC- or AC operated Wheatstone bridges as described previously [9]. Unless otherwise mentioned, the AC measurements in pulse radiolysis were carried out at a measuring frequency of 10^5 Hz.

Solutions of free deoxyribonucleic acid (H^+DNA) were obtained by passing solutions of the sodium salt of DNA through a column containing a strongly acidic cation exchanger (Merck, Type I).

Pulse irradiation was carried out with electron pulses (duration 0.4–2.0 μs) of 2.8 MeV generated by a Van de Graaf accelerator (High Voltage Engineering).

^{23}Na-NMR measurements were performed with a Bruker WH 270 FT NMR spectrometer. With 15 μs pulses (ca. 60°) a signal to noise ratio of ~ 20 was achieved after 900 runs with a delay time of 12 s between runs with unirradiated DNA of 260 mg l^{-1}. In all NMR experiments the solvent was H_2O/D_2O in the ratio 1:1 by volume.

Results

^{60}*Co-γ Irradiations*

Conductivity measurements

Aqueous, N_2O saturated non membrane filtered and not heat treated solutions of DNA (260 mg l^{-1}, initial molecular weight $\bar{M}_w = 5 \times 10^6$ g mol^{-1}) were irradiated at 20 °C with ^{60}Co-γ rays (dose rate 0.3 J kg^{-1} s^{-1}). The conductivity before ($\varkappa_0$) and after irradiation ($\varkappa$) was recorded as a function of dose (range $0-2 \times 10^3$ J kg^{-1}), see circles in Fig. 2; the constant value $\varkappa_{max}$ denotes the conductivity measured at 2.0×10^3 J kg^{-1}. It is seen, that the conductivity first decreases by ~ 0.5% at doses $\leqq 30$ J kg^{-1} and then increases drastically. The decrease of conductivity especially pronounced at low doses and high DNA concentrations is possibly the result of crosslink formation and will be discussed in a forthcoming paper. At doses $\geqq 4 \times 10^2$ J kg^{-1} the increase becomes less pronounced until a plateau $\varkappa_\infty$ is approached for $D \geqq 1.5 \times 10^3$ J kg^{-1}. The pH value of the solution after absorption of 1.5×10^3 J kg^{-1} shifted from 7.4 to 6.7, whereas the conductivity increased from $\varkappa_0 = 4.1 \times 10^{-5}\ \Omega^{-1}\ cm^{-1}$ to $\varkappa_{max} = 7.1 \times 10^{-5}\ \Omega^{-1}\ cm^{-1}$ in the same dose range. The pH shift accounts for an increase of conductivity of $\sim 10^{-8}\ \Omega^{-1} cm^{-1}$ only and hence cannot be responsible for the conductivity increase observed (equivalent conductances of 325 and 178 $cm^2\ \Omega^{-1}\ mol^{-1}$ were used for H^+ and OH^- at 20 °C in the calculation [25]). A similar increase of

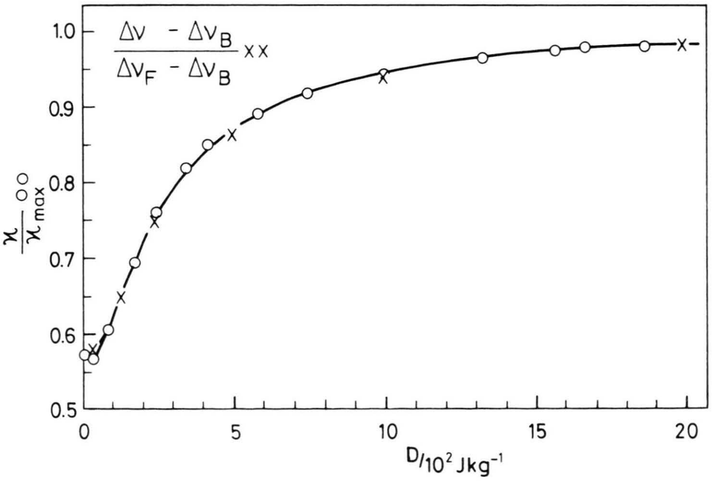

Fig. 2. Relative increase of conductivity $\varkappa$ (○○○) and relative decrease of ^{23}Na NMR signal linewidth (××) as a function of dose measured after ^{60}Co-γ irradiation (dose rate 0.3 J kg^{-1} s^{-1}) in N_2O saturated solutions of non-sonicated and non-membrane filtered DNA (260 mg l^{-1}) at 20 °C (○○) and 27 °C (××). Initial pH of the solutions = 7.4 ± 0.2. $\varkappa$ and $\Delta\nu$ are the conductivity and the linewidth observed with solutions at the respective doses. $\varkappa_{max}$ is the plateau conductivity at doses $\geqq 1.5 \cdot 10^3$ J kg^{-1}, $\Delta\nu_F$ and $\Delta\nu_B$ denote the relative linewidth of the free and bound sodium ions. $\Delta\nu_F = 12.3$ Hz and $\Delta\nu_B = 90.3$ Hz were used for the construction of the curve in the figure (see text).

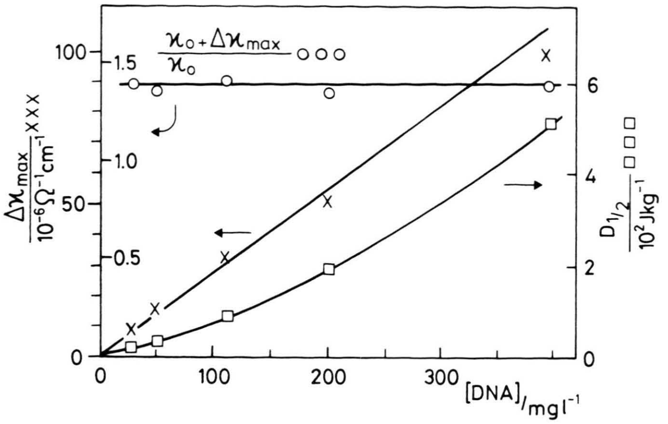

Fig. 3. Concentration dependence observed with aqueous N_2O-saturated sonicated and heat treated DNA solution at room temperature of (i): the maximum conductivity increase $\Delta\varkappa_{max}$ obtainable with increasing dose (××), (ii): the relative conductivity increase $(\varkappa_0 + \Delta\varkappa_{max})\,\varkappa_0^{-1}$ (○○), and (iii): the irradiation dose $D_{1/2}$ (□□)(the dose at which the conductivity increase has reached $\Delta\varkappa_{max}/2$).

conductivity accompanied by small pH changes was observed previously with polyuridylic acid [9] and was shown to be due to the liberation of electrostatically bound counterions as a consequence of decreasing chain length.

With sonicated and thermally denatured DNA solutions the initial decrease of conductivity was no longer observed, but apart from that the dependence of conductivity on irradiation dose was similar, *i.e.* the conductivity increased with increasing dose from $\varkappa_0$ to a plateau value $\varkappa_{max}$ by a maximum conductivity increase $\Delta\varkappa_{max} = \varkappa_{max} - \varkappa_0$. The irradiation dose at which the conductivity increase $\Delta\varkappa$ has reached $\Delta\varkappa_{max}/2$ is called $D_{1/2}$. The dependence on DNA concentration of $\Delta\varkappa_{max}$, of the relative conductivity increase $(\varkappa_0 + \Delta\varkappa_{max})/\varkappa_0$ and of $D_{1/2}$ is presented in Fig. 3 (pH = 7.4, 22 °C, starting $\bar{M}_w = 7 \cdot 10^5$ g mol^{-1}). It is shown that $\Delta\varkappa_{max}$ is a linear function of the concentration and that the fraction $\varkappa_{max} \cdot \varkappa_0^{-1} = 1.39$ does not depend on concentration. The curve for $D_{1/2}$ is bent upwards, *i.e.* at higher concentrations of DNA relatively higher doses have to be applied in order to achieve $\Delta\varkappa_{max}/2$. As can be deduced from Fig. 3, the conductivities $\varkappa_0$ and $\varkappa_{max}$ (not shown in the figure) are found to have an approximate linear relationship to the concentration of DNA in the range 25 mg l^{-1} < [DNA] < 250 mg l^{-1} (corresponding to 0.7×10^{-4} M < [DNA] < 7×10^{-4} M with concentration expressed in molarities of nucleotides). On using 3.15×10^{-4} M DNA, $\varkappa_0 = 2.20\times10^{-5}\ \Omega^{-1}\,cm^{-1}$ and $\varkappa_{max} = 3.05\times10^{-5}\ \Omega^{-1}\,cm^{-1}$ was measured at 22 °C.

^{23}*Na-NMR measurements*

In Fig. 4 a ^{23}Na-NMR spectrum is shown for a sample containing 260 mg l^{-1} unirradiated DNA measured under experimental conditions described in the figure legend. Under these conditions a linewidth of $\Delta\nu = 46 \pm 4$ Hz was found ($\Delta\nu$ = linewidth at half height). This linewidth is independent of the concentration of DNA in the range 2.8×10^3 mg l^{-1} ≧

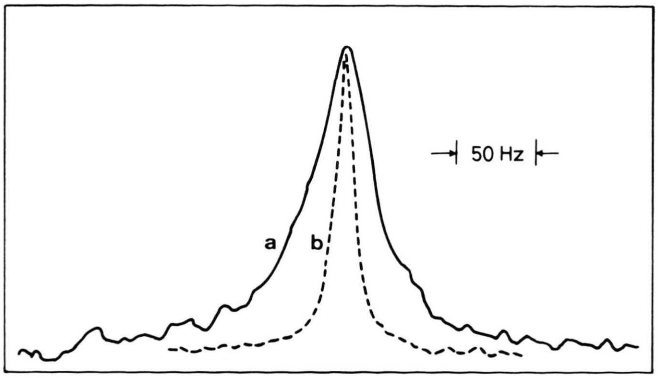

Fig. 4. ^{23}Na-NMR spectra of non heat-treated DNA (260 mg l^{-1}) obtained at 27 °C, pH = 7.4 after accumulation of 900 pulses of 15 µs duration (≈60°) with 12 s delay time. Solvent: H_2O/D_2O = 1/1 by volume. a) unirradiated solution, b) after absorption of 2×10^3 J · kg^{-1}.

$[\mathrm{DNA}] \geqq 28$ mg l^{-1}. From the spectrum of a mixture of 2′-deoxyadenosine 5′-monophosphate and 2′-deoxyguanosine 5′-monophosphate a value of $\Delta\nu = 12.3 \pm 1$ Hz was obtained under otherwise equal experimental conditions (10^{-3} M NaCl yielded $\Delta\nu = 10.5 \pm 1$ Hz).

After irradiation of the DNA solutions, the observed linewidth became smaller with increasing dose and appears to approach a limiting linewidth of $\Delta\nu = 13.3 \pm 1$ Hz for doses $\geqq 1.5 \times 10^3$ J kg^{-1}. The dose dependence of the decrease of linewidth and of the conductivity increase described above occurred in the same dose range. To analyze the relation between linewidth and conductivity in more detail, it is assumed that the Na^+ ions stay in either of two distinct environments termed "bound" and "free" [14, 15].

Each of the states is characterized by its own relaxation rate, *i.e.* by one NMR-linewidth abbreviated with $\Delta\nu_\mathrm{B}$ ("bound") and $\Delta\nu_\mathrm{F}$ ("free") whereby generally $\Delta\nu_\mathrm{B} > \Delta\nu_\mathrm{F}$. The fraction α of the free counterions is then related to the observed linewidth by Eq. (1).

$$\alpha = \frac{\Delta\nu - \Delta\nu_\mathrm{B}}{\Delta\nu_\mathrm{F} - \Delta\nu_\mathrm{B}}. \qquad (1)$$

Eq. (1) is readily derived from relations presented in the literature [14, 15]. For $\Delta\nu_\mathrm{F}$ the value found with the monomeric nucleotide compounds is used. $\Delta\nu_\mathrm{B}$ is calculated from expression (1) on substitution of a value for α which is obtained from conductivity measurements. Eq. (2) defines the dependence of α on conductivity $\varkappa$ and was used with $\varkappa = \varkappa_0$.

$$\alpha = \frac{\varkappa}{\varkappa_\mathrm{max}}. \qquad (2)$$

The dependence on dose of both expressions then makes possible a direct comparison of the action of ^{60}Co-γ rays on the DNA solutions with respect to ^{23}Na-NMR linewidth and to conductivity. The result is presented in Fig. 2. The close similarity of both curves in the figure strongly suggests that the conductivity increase is caused by the transition of Na^+ counterions from the bound to the free state.

Pulse radiolysis

All DNA solutions used in experiments described in this section were subjected to sonication for 10 min and afterwards to thermal degradation at 90 °C for 5 min. After this treatment the molecular weight of DNA was typically $\bar{M}_\mathrm{w} = 5 \pm 1 \times 10^5$ g mol^{-1} as determined by light-scattering measurements. Unless otherwise stated the pH was 7.4 ± 0.2, the temperature 20 ± 2 °C, the measuring frequency 10^5 s^{-1} and the solutions were $\mathrm{N_2O}$ saturated. Figure 5a shows the time dependence of the conductivity change observed in the 0.1 s time range after a 1 µs electron pulse of 10 J kg^{-1} at 90 mg l^{-1} DNA. The conductivity increases rapidly within ≈ 0.02 s after the pulse (Fig. 5a). This fast component of the increase will be referred to as "first". After 0.02 s there is a second increase, which is complete after ≈ 0.5 s, Fig. 5b. In some experiments the conductivity remained constant after this second increase for $\geqq 20$ s. In these experiments the second component accounted for $\approx 25\%$ of the total conductivity increase, the latter being 0.38×10^{-6} Ω^{-1} cm^{-1} after 10 J kg^{-1}.

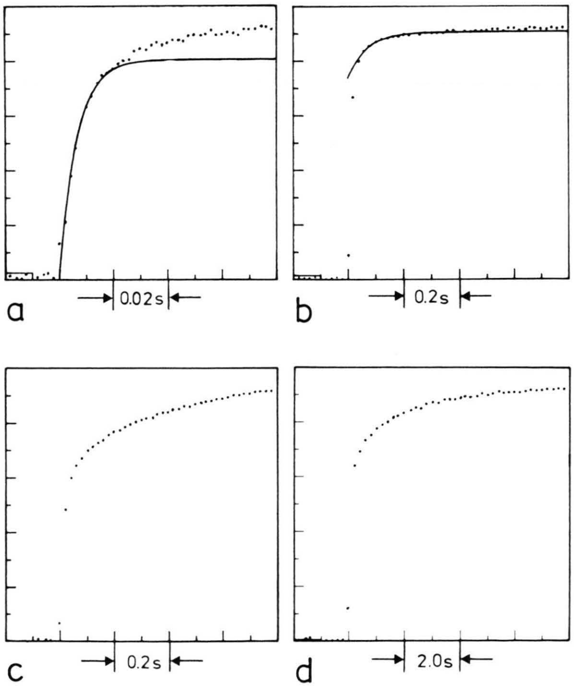

Fig. 5. Computer plots of the time dependence of conductivity (in rel. units) as observed in pulse radiolysis of aqueous, $\mathrm{N_2O}$-saturated solutions at 20 °C, pH = 7.4 of sonicated and heat-treated DNA (140 mg l^{-1}, $\bar{M}_\mathrm{w} = 4 \times 10^5$ Dalton) after a 1 µs pulse of 20 J kg^{-1} for various time ranges (full scale): a) 0.1 s, b) 1.0 s, c) 1.0 s, d) 20.0 s. The points (···) are the experimentally determined conductivity increase, the solid lines are first order curve fits ($k = 150$ s^{-1} in figure 5a, $k = 15$ s^{-1} in figure 5b).

In other experiments we found in addition a third increase which was much slower than the second one described above and which ceased only after 10 to 20 s (Figs. 5c + d). Also in the latter experiments the total conductivity increase after 20 s was $0.40 \times 10^{-6}\,\Omega^{-1}\,\mathrm{cm}^{-1}$ on the average and hence very similar to that in the absence of these very slow components. In some experiments the second and third parts of the increase together amounted to up to 50% of the total increase. Measurements at times longer than 20 s were difficult to carry out because of stability problems with the base line (the conductivity of the unirradiated solution). However, the results of a few measurements indicate that the conductivity remains constant at least up to 1.5 min after pulse.

No significant difference was found when the experiments were carried out 15 min or 24 h after the denaturation process provided the solutions were kept at room temperature.

In those cases, where the third component was comparatively small, the conductivity increase could be fitted to the sum of two first order exponentials with rate constants of $k = \ln 2 \cdot t_{1/2}^{-1} = (1.5 \pm 0.2) \times 10^2\,\mathrm{s}^{-1}$ and $k = (15 \pm 2)\,\mathrm{s}^{-1}$ (Figs. 5a and 5b), respectively. The first (fast) component made up about 75% of the total conductivity increase. Measurements 0.08 s after pulse at room temperature include the first component and roughly 70% of the second component. For kinetic analysis the first and the second components were separated (see Fig. 5a). The separation of the first and the second components is difficult at higher and lower pH values due to lower signals and to higher intrinsic conductivity of the solutions. When the second and third components contributed 50% to the total conductivity increase a best fit to a single exponential was obtained with $k = (1.1 \pm 0.2) \times 10^2\,\mathrm{s}^{-1}$ for the first part. It was not possible to fit the third component in the 20 s time range to a single exponential function. It is interesting that the observed rate constants for the first increase of conductivity are in the same range as values obtained by Schnabel and his group [16] by means of time dependent light scattering measurements. The k values of the plateau at higher doses in Fig. 9 of Schnabel's paper is in agreement with our value despite the fact that Schnabel's measurements are performed at much higher ionic strength ($\mathrm{NaCl} = 10^{-2}\,\mathrm{M}$).

Dependence on measuring frequency and dose rate

$\Delta\varkappa$ was found to be 0.38, 0.44 and $0.48 \times 10^{-6}\,\Omega^{-1}\,\mathrm{cm}^{-1}$ at frequencies ν of 2×10^5, 2×10^3 and 10^2 Hz in the absence of the third component measured 0.08 s after pulse with DNA solutions of $140\,\mathrm{mg\,l^{-1}}$. With a DC-operated Wheatstone bridge ($\nu = 0$) a value of $\Delta\varkappa = 0.40 \pm 0.05 \times 10^{-6}\,\Omega^{-1}\,\mathrm{cm}^{-1}$ was measured 0.08 s after pulse. This shows that the influence of the frequency is small. In the ^{60}Co-γ experiments where the measuring frequency was 2×10^3 Hz we obtained a total conductivity increase of $\Delta\varkappa = 0.52 \times 10^{-6}\,\Omega^{-1}\,\mathrm{cm}^{-1}$. The value obtained in pulse radiolysis is similar suggesting the absence of pronounced dose rate effects on the yields, since in pulse radiolysis the dose rate is $\sim 10^7$ times higher than under ^{60}Co-γ irradiation conditions. The rate of the conductivity build-up did not depend on the measuring frequency in the range $10^4 - 2 \times 10^5$ Hz. At frequencies below 10^4 Hz the half life of the increase which could be measured is limited mainly by the electronic rise time of the measuring apparatus and hence the rate of conductivity build up in the solution cannot be determined.

Dependence on concentration of DNA and dose per pulse

Fig. 6 presents the increase of conductivity $\Delta\varkappa$ measured 0.08 s after the pulse as a function of the absorbed dose per 1 µs electron pulse at various concentrations of DNA. It is seen that the slopes of the curves in Fig. 6 become smaller with increasing dose (dashed lines) and therefore the yield of conductivity per $10\,\mathrm{J\,kg^{-1}}$ decreases with increasing dose. This decrease is more pronounced at lower concentations of DNA (see Fig. 6) and is explained to be mainly due to incomplete scavenging of OH radicals by DNA *i.e.* at low concentrations of DNA the OH radicals undergo combination rather than reaction with DNA. The solid line in Fig. 6 with a slope corresponding to an conductivity increase of $0.4 \times 10^{-6}\,\Omega^{-1}\,\mathrm{cm}^{-1}$ per $10\,\mathrm{J\,kg^{-1}}$ is an overall representation of many points in the diagram at which the effect of incomplete scavenging is small.

The rate of the first conductivity increase was $160 \pm 20\,\mathrm{s}^{-1}$ at 20 °C throughout the measurements described in Fig. 6. It did depend neither on DNA concentration (range $7-40\,\mathrm{mg\,l^{-1}}$) nor on the dose per pulse (range $2-20\,\mathrm{J\,kg^{-1}}$) with the exception of an $\approx 20\%$ increase at the highest concentration of

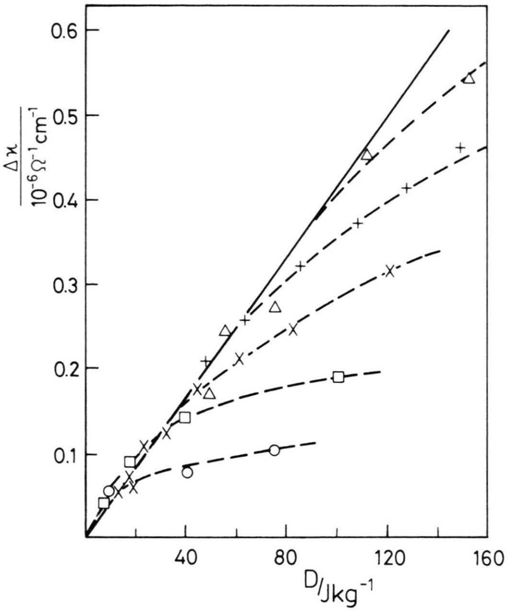

Fig. 6. Dependence of the size of the conductivity increase $\Delta\varkappa$ on dose per pulse at various concentrations of sonicated and heat-treated DNA observed in pulse radiolysis 0.08 s after the pulse (N_2O saturated solutions at pH = 7.4 and 20 °C, DC method). DNA concentration: △△ = 140 mg l^{-1}; ++ = 70 mg l^{-1}; ×× = 35 mg l^{-1}; □□ = 18 mg l^{-1}; ○○ = 7 mg l^{-1}.

DNA used. This provides evidence, that the first and rapid component of the conductivity increase is governed by first order reactions.

pH dependence

The pH dependence ($4.3 < \mathrm{pH} < 10.3$) of the yield of conductivity $\Delta\varkappa$ 0.08 s after pulse as well as that of the rate constant k of the first component are presented in Fig. 7. $\Delta\varkappa$ passes through a maximum at pH = 7.5 and decreases towards the alkaline and acidic pH range. A decrease with increasing pH was also observed with poly U [9]. This was explained by the effect of ionic strength of the added potassium hydroxide and by the influence of the pK value of the base. By analogy we consider a similar explanation to apply in the case of DNA.

An ionic strength effect of the added perchloric acid contributes also to the decrease of $\Delta\varkappa$ with decreasing pH. With poly U an ionic strength effect at lower pH values was also observed (smaller increase of $\Delta\varkappa$ as expected [9]). In contrast to poly U, DNA exhibits a considerable buffer capacity at acidic pH values because of the presence of $-NH_2$ groups at the cytosine and adenosine moiety of DNA [17]. Hence we had to add much more $HClO_4$ to obtain a desired pH value in the case of DNA compared to poly U. The effect of ionic strength at fixed pH is thus increased correspondingly. A consequence of the protonation of the bases is partial neutralisation of the charges of the polyanionic chain which may reduce the fraction of counterions condensed at the polymer. The latter effect could also contribute to the decrease of $\Delta\varkappa$ because in this case fewer counterions are expected to be released after irradiation. A similar but still more pronounced decrease of $\Delta\varkappa$ with decreasing pH was observed with poly C, which contains a $-NH_2$ group at every base moiety [17].

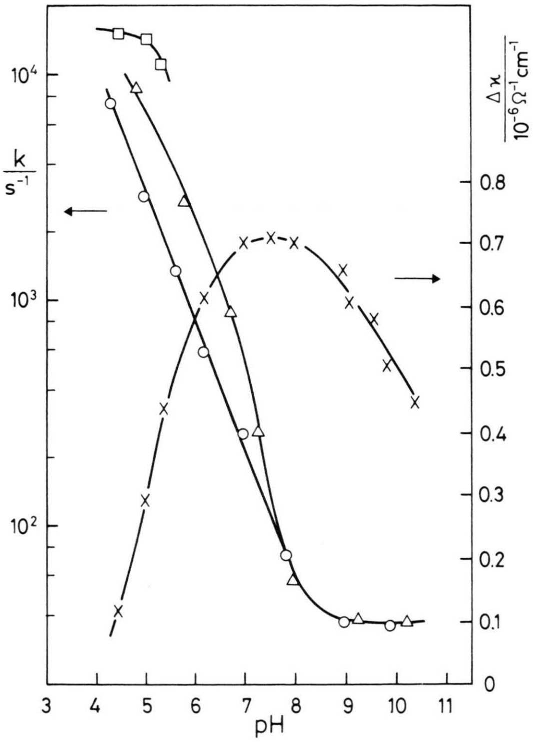

Fig. 7. pH Dependence of the conductivity increase $\Delta\varkappa$ (right ordinate) observed 0.08 s after 1 µs electron pulses and of the logarithm of the rate constant k (left ordinate) of the first component of the conductivity increase (N_2O saturated aqueous solutions of sonicated and heat denatured DNA at 20 °C). ×× refers to the right ordinate and was measured after a pulse of 20 J kg^{-1} with the DC method. The other symbols refer to the left ordinate and were measured at 10^5 Hz. DNA concentrations: ×× and ○○ = 140 mg l^{-1}; △△ = 17 mg l^{-1}; □□ refers to metal ion free DNA (H^+DNA), whereby pH variation was achieved by dilution.

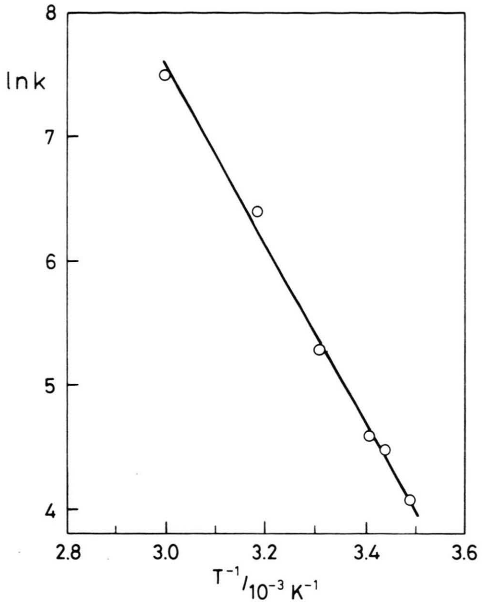

Fig. 8. Arrhenius plot (ln k *versus* T^{-1}) of the first component of conductivity increase measured with the DC method (N_2O-saturated aqueous solutions of sonicated and heat-denatured DNA (70 mg l^{-1}) after 1 µs electron pulses of 20 J kg^{-1} at pH 7.6).

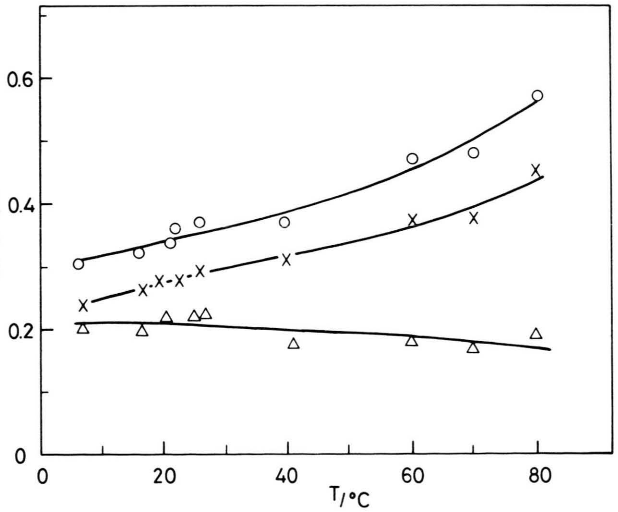

Fig. 9. Dependence of the size of the conductivity increase $\Delta\varkappa$ on the temperature of sonicated and heat treated DNA solutions (90 mg l^{-1}) after 1 µs electron pulses of 10 J kg^{-1}. △△ = after the first initial increase, ×× = after 0.8 s, ○○ = after 8.0 s (pH 7.4, N_2O saturated, measuring frequency 10^5 Hz, sonicated and heat-denatured solutions). The $\Delta\varkappa$ values are already corrected for the increase of the equivalent conductances with increasing temperature, see text.

The rate constant k of the first conductivity build-up is constant at pH > 9.0 ($k = 38\ s^{-1}$) but shows a marked increase from pH 7.0 to 4.3 (Fig. 7). The greatest value of the rate constant measured with 140 mg l^{-1} DNA at 20 °C amounted to $7.5 \times 10^3\ s^{-1}$ at pH = 4.3, the smallest pH which allowed conductivity measurements. At 17 mg l^{-1} DNA, the increase of the rate constant with decreasing pH is steeper in the beginning (pH 8 to 6.5) compared to 140 mg l^{-1} DNA and afterwards (pH 6.5 to 4.3) slightly less pronounced (Fig. 7). With isoionic solutions of potassium-free DNA (H^+DNA) the pH values were adjusted by dilution of the H^+DNA solutions. Around pH = 4.5 there is a small plateau (Fig. 7) at which the rate constant is independent of pH. At this plateau the rate constant is $k = 1.2 \times 10^4\ s^{-1}$ at 20 °C. The experimental errors in the rate constants at pH values around 4.5 may be as high as ±25% because the increase of conductivity at that pH is small. At near neutral pH the errors are approximately ±10%. The rate constant was found to be independent of dose per pulse (range 2–20 J kg^{-1}) in the whole pH range studied. This shows that the conductivity increase is due to first order processes.

Dependence on temperature

The influence of temperature on the rate constant of the first conductivity increase was studied in the range 5 °C < T < 60 °C at pH = 7.3 and 70 mg l^{-1} DNA. Plotting ln k *versus* T^{-1} a straight line was obtained (Fig. 8). The calculated activation energy was $E_a = 13$ kcal mol^{-1}, the frequency factor $A = 5 \times 10^{11}\ s^{-1}$. The corresponding values for the second part of the conductivity increase obtained from solutions not showing the third component were $E_a = 13$ kcal mol^{-1} and $A = 7 \times 10^{10}\ s^{-1}$.

The dependence of $\Delta\varkappa$ on temperature measured for the first component as well as 0.8 s and 8 s after pulse are presented in Fig. 9 for heat treated solutions which show a considerable contribution of the third component. A correction for the influence of the temperature on the equivalent conductances is already included in Fig. 9, assuming the same temperature dependence for the poly anion as for Na^+. The size of the first (Fig. 9) and second component of the conductivity increase remains nearly constant with temperature. This holds also for solutions with negligible contribution of the third component. Therefore the ratio of 75 : 25 for the first and second components did not depend on temperature (range $9\,°C < T < 80\,°C$). The contribution of the third component however increased significantly with increasing temperature (Fig. 9). The activation energy of the third part can only be estimated to be $\leqq 5$ kcal mol^{-1}.

Dependence on addition of p-benzoquinone (pBQ)

When pBQ is added in small amounts to a solution of DNA (140 mg l^{-1}) it is observed that the initial rate of the first conductivity build-up increases: the rate constant k is a linear function of the concentration of pBQ (Fig. 10). In the experiments $\Delta\varkappa$ decreased on addition of pBQ. Because of its small contribution to $\Delta\varkappa$ the influence of pBQ on the rate of the second component could not be studied.

Dependence on chain length

N_2O saturated solutions of sonicated DNA ($\bar{M}_w = 4 \times 10^5$ Dalton, concentration 140 mg l^{-1}) were preirradiated at the ^{60}Co-γ source with 150 and 300 J kg^{-1} and afterwards the conductivity increase was measured under pulse radiolysis conditions at doses of 16 J kg. The size of the conductivity increase $\Delta\varkappa$ measured 0.08 s after pulse was found to decrease with increasing dose of preirradiation from $0.32 \times 10^{-6}\,\Omega^{-1}\,cm^{-1}$ with no preirradiation to $0.20 \times 10^{-6}\,\Omega^{-1}\,cm^{-1}$ after 150 J kg^{-1} and $0.10 \times 10^{-6}\,\Omega^{-1}\,cm^{-1}$ after 300 J kg^{-1}. The rate constant of the first conductivity build-up in these experiments increased from 110 s^{-1} to 140 s^{-1} and 220 s^{-1} with zero, 150, and 300 J kg^{-1} preirradiation dose. Taking a G value for the main chain scission of $G(\mathrm{ssb}) = 0.55$ for DNA in N_2O saturated solution [3], the number average degree of polymerisation is calculated to decrease from 570 to 44 and 24 after preirradiation with the doses cited above.

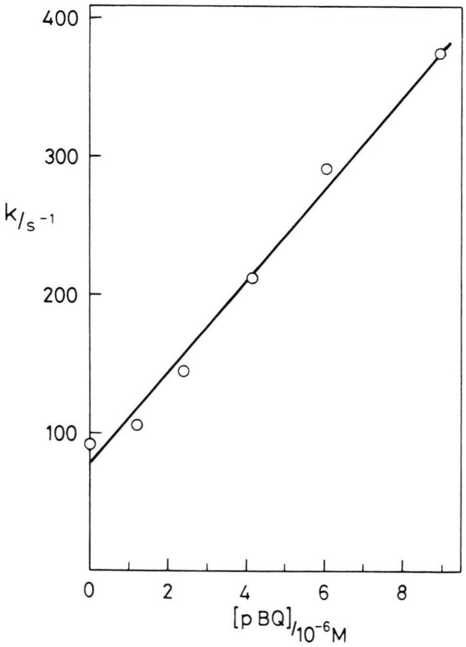

Fig. 10. Rate constants of the first increase of conductivity as a function of the concentration of added p-benzoquinone (pBQ), (N_2O saturated aqueous solutions of sonicated and heat-denatured DNA (70 mg l^{-1}) at pH 7.6, 18 °C and 10^5 Hz measuring frequency). The dose was increased successively with increasing concentration of pBQ from 3 J kg^{-1} to 6 J kg^{-1} in order to compensate for smaller signals at higher concentrations of pBQ.

Discussion

Structure of the DNA

Commercially available calf thymus DNA dissolved at 0 °C in distilled water shows a hyperchromic effect of ~ 32% (see Fig. 1). Since this value is similar to that observed in solutions with higher ionic strength in which case it is explained to be a consequence of a "melting" of the DNA (transition from the double stranded to the single stranded DNA) [14], we conclude that DNA dissolved at low temperatures in distilled water is present in double stranded form and is denatured with increasing temperature. Membrane filtration of such a double stranded DNA solution at room temperature leads to a solution which shows a hyperchromic shift of ~ 8% only (Fig. 1). The shape of the curve has, however, similarities with the melting curve of a double stranded DNA reflecting a cooperative effect. A solution of DNA heated for 5 min at 90 °C and cooled down to room temperature also has a hyperchromic shift of about 8%. However, the shape of this curve resembles that for the dissociation of base stacking (non-cooperative effect). The results indicate that the heat treated solutions contain only single stranded DNA. Since the hyperchromic shift of such a solution does not change with time for days, the solution is apparently stable enough for studies on the DNA in the single stranded form.

The form of the DNA in the membrane-filtered but not heat-treated solution is of some interest.

One explanation may be that the DNA is basically still in the double stranded form, however, large parts of the double strands are already "molten". As will be shown in a forthcoming paper the membrane filtered solution shows a completely different behaviour under irradiation than the membrane filtered and heat treated solution.

Origin of the conductivity increase

A number of theoretical approaches have been undertaken in the past to describe electrostatic binding of counterions to polyions in course of which two models were introduced. One, which is often referred to as the "two site model" was put forward mainly by Oosawa [18] and Manning [10–12]. The other ones originate from attempts to solve the Poisson-Boltzmann equation under certain boundary conditions [19–23]. In order to permit a simple treatment we will discuss our results mainly in terms of the two site model. According to this model, which has been developed for infinitely long chains the counterions can be present in two states, one in which they do not contribute to the electrical conductivity (the "bound" one), the other one in which they contribute to the conductance (the "free" one). The fraction of bound counterions decreases with decreasing chain length of the polymer, as was found earlier by Schindewolf for polyphosphates [13]. Recently this was observed also for polyuridylic acid (poly U) [9] and polycytidylic acid (poly C) [17]. According to calculations of Woodbury *et al.* [24] this dependence on chain length is a result of the increasing influence of end effects as the chain length decreases.

Electron and γ-irradiation of aqueous DNA solutions leads to single strand breaks in the substrate with a G value of around 0.55 [3]. Hence the chain length decreases on irradiation and the conductivity is expected to increase. The similarity of the two curves in Fig. 2 provides evidence that the conductivity increase is indeed caused by the liberation of bound counterions. Furthermore the agreement of rate constants obtained by conductivity on one hand and by light scattering from Schnabel's group (*cf.* Fig. 9 in [16]) on the other hand also shows that the conductivity changes are related to the formation of chain breaks.

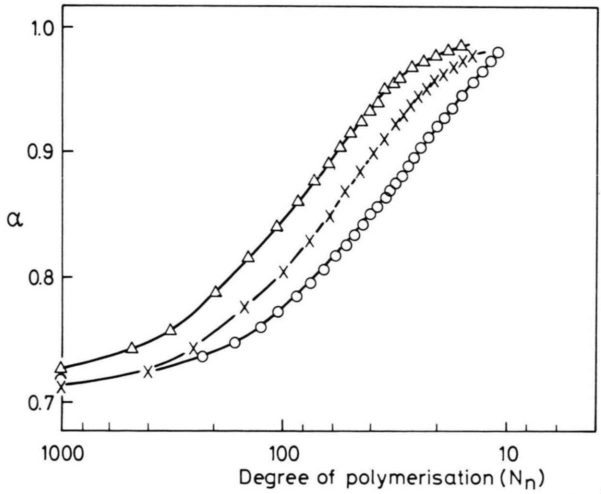

Fig. 11. Dependence of the value $\alpha = \varkappa \cdot \varkappa_{\max}^{-1}$ on the number average degree of polymerisation $\bar{N}_n$ observed with aqueous heat-denatured and sonicated DNA solutions (pH 7.4, T 21 °C) of three different concentrations calculated on the assumption of a constant G value for single strand break formation of $G(\text{ssB}) = 0.55$, [3]. DNA concentrations: ○○ 400 mg l^{-1}, ×× = 115 mg l^{-1}, △△ = 28 mg l^{-1}.

Degree of dissociation and equivalent conductance of single stranded DNA

The conductivity of DNA solutions increases with increasing dose from $\varkappa_0$ to $\varkappa_{\max}$ (Fig. 3). We interpret $\varkappa_{\max}$ as representing the conductivity of DNA solutions in which all bound counterions are set free as a result of sufficient reduction of chain length. Assuming that the equivalent conductances of the ions involved are the same before and after irradiation $\varkappa \cdot \varkappa_{\max}^{-1}$ can be interpreted to be the degree of dissociation α of DNA. The value $\varkappa_0 \cdot \varkappa_{\max}^{-1} = 0.72$ would then be the degree of dissociation of high molecular-weight DNA. This value is higher than the value derived by Manning ($\alpha = 0.56$). Our value (which was measured in salt free solution) has to be considered a crude approximation however, since we do not know whether or not (i) the contribution of screening effects and (ii) the equivalent conductance of the polyanion of DNA ($\lambda(\text{DNA}^-)$) are independent of chain length ($\varkappa_0$ is measured at degrees of polymerisation of $\bar{N}_n \sim 1000$, $\varkappa_{\max}$ at $\bar{N}_n \sim 10$, Fig. 11). From the $\varkappa_{\max}$ value given in the results a total equivalent conductance for DNA of $\lambda(\text{DNA}) = 97\ \text{cm}^2\,\Omega^{-1}\,\text{mol}^{-1}$ is calculated, which after subtraction of the value for Na$^+$ ($47\ \text{cm}^2\,\Omega^{-1}\,\text{mol}^{-1}$,

[25]) leads to $\lambda(\text{DNA}^-) = 50\ \text{cm}^2\,\Omega^{-1}\,\text{mol}^{-1}$ for the equivalent conductance of the DNA anion with respect to the monomer at 22 °C. This value refers to DNA with a low degree of polymerisation of $\bar{N}_\text{n} \sim 10$ (Fig. 11).

For the anion of the mononucleotide 2′-deoxyadenosine-5′-monophosphate we found $\lambda = 35\ \text{cm}^2\,\Omega^{-1}\,\text{mol}^{-1}$.

The dependence of α on chain length

Using $G(\text{ssb}) = 0.55$ [3] we calculated for each dose a number average degree of polymerisation assuming poissonian molecular weight distribution of DNA strands. Thus it was possible to calculate the dependence of $\alpha = \varkappa \cdot \varkappa_\text{max}^{-1}$ on the average chain length of DNA. Fig. 11 shows results obtained with three different DNA concentrations. It is seen that α approaches a constant value with respect to the number average chain length for high degrees of polymerisation ($\bar{N}_\text{n} \geqq 500$). For $200 \gtrsim \bar{N}_\text{n} \gtrsim 20$ there is a steep increase in α towards unity (half value of α is reached at $\bar{N}_\text{n} = 60$ for $[\text{DNA}] = 115\ \text{mg}\,\text{l}^{-1}$). Below $\bar{N}_\text{n} \lesssim 20$ DNA has lost nearly all its polymeric properties with respect to counterion association. The loss of polyelectrolytic behaviour is shifted to smaller chain lengths at higher concentrations of DNA (Fig. 11). Similarly $D_{1/2}$ is relatively greater at higher concentrations of DNA, see Fig. 3. We interpret these findings to result from the increased ionic strength of the solution at higher concentrations of DNA. An ionic strength effect of the same order of magnitude was also observed after addition of a corresponding amount of NaClO_4. From Fig. 11 the average number of sodium ions $\bar{N}(\text{Na}^+)$ which are liberated per single-strand break can be calculated (Fig. 12). It is seen that $\bar{N}(\text{Na}^+)/\bar{N}(\text{ssb})$ is constant with respect to the degree of polymerisation $\bar{N}_\text{n}$ at high values of $\bar{N}_\text{n}$ and is higher at lower concentrations of DNA. The influence of DNA concentration on $\bar{N}(\text{Na}^+)/\bar{N}(\text{ssb})$ is the effect of ionic strength on ion association described above. At $28\ \text{mg}\,\text{l}^{-1}$ DNA $\bar{N}(\text{Na}^+)/\bar{N}(\text{ssb}) = 15$ for $400 > \bar{N}_\text{n} > 300$ from which one calculates a G value of sodium ion formation of $G(\text{Na}^+) = 15 \times G(\text{ssb}) = 8.3$.

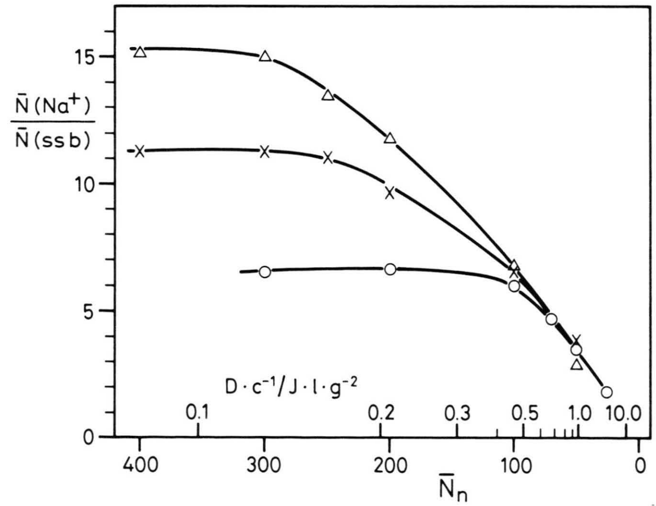

Fig. 12. Dependence of the average number of sodium ions liberated per single-strand break ($\bar{N}(\text{Na}^+)/\bar{N}(\text{ssb})$) on (i) the average degree of polymerisation $\bar{N}_\text{n}$ before strand break and (ii) on the irradiation dose D per DNA concentration c. The points were calculated from the α values of Fig. 11. Experimental conditions and symbols as in Fig. 11.

Rate-determining step of the conductivity increase

For the rate-determining step of the conductivity increase five possibilities are discussed in more detail.

Firstly, the rate of the reaction of OH radicals with DNA may be rate determining. However, this is not the case since the reaction of OH with DNA has a rate constant of $k \approx 10^8\ \text{s}^{-1}$ from which half-lifes shorter than $t_{1/2} \approx 10^{-5}\ \text{s}$ are calculated. They are more than a hundred times shorter than those measured for the conductivity increase at pH 7.4.

Secondly, the rate of the strand break formation may be fast and the rate-determining step is the establishment of the new ionic equilibrium. The following facts speak against this hypothesis. The rate-determining step has an activation energy of $13\ \text{kcal mol}^{-1}$. This value is much larger than expected for the liberation of sodium or potassium ions which are bound only electrostatically. Furthermore from the interpretation of the frequency dependence of the dielectric constant of DNA solutions it has been concluded that the metal ions move very fast along the chain and have relaxation times in the order of 10^{-5} s [26]. This clearly shows that the counterions are not firmly bound to the DNA. The rate of the establishment of ionic equilibria for ions not bound by homopolar bonds should therefore occur at diffusion-controlled rates which are much faster than the rates observed for the conductivity increase.

The *third* possibility is that the rate-determining step may be the diffusive separation of the broken DNA strands. However, the measured activation energy of 13 kcal mol^{-1} is apparently too high to be compatible with this hypothesis. Furthermore the diffusion hypothesis would not easily explain the large dependencies of the rate of the conductivity increase observed on changing the pH (Fig. 7) and on adding small amounts of pBQ (Fig. 10).

As a *fourth* possibility the rearrangement of the DNA structure after the reaction with OH radicals may be rate-limiting. However, it is difficult to see what kind of rearrangement except for splitting this could be since in solutions of low ionic strength the DNA has a rod-like structure because of the repelling forces of the negative charges. Further arguments against this and the other possibilities already discussed are presented in the next paragraph.

The *fifth* possibility would be a chemical cleavage reaction of DNA radicals as the rate-determining step which leads to the formation of strand breaks. This suggestion is supported by the effect of pBQ as a scavenger on the rate of the fast part of the conductivity increase. If the scavenger would scavenge only OH radicals and prevent them from producing strand breaks the rate of the conductivity increase should not be influenced by the concentration of the scavenger. However, if the scavenger reacts with DNA radicals thus preventing them from producing strand breaks (and hence conductivity), the rate constant of the conductivity increase should be a linear function of the scavenger concentration [S], equation (3) [9], as is indeed observed (Fig. 10).

$$k_{\mathrm{obs}} = k_1 + k_2\,[\mathrm{S}]\,. \tag{3}$$

In equation (3) k_1 and k_2 denote the rate constants for strand break formation of DNA radicals and for the reaction of pBQ with these DNA radicals, respectively. From the slope of the straight line in Fig. 12, k_2 is calculated to be $3.0 \times 10^7\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$ (± 30%). The dependence of k_{obs} on [S] provides evidence that at least the first component of the conductivity increase is governed by the chemical reaction leading to strand breaks.

As to the second component of the kinetics all arguments enumerated before hold with the exception of the argument derived from the effect of pBQ, since the experimental data could not be fitted to equation (3) with certainty. We feel however that also the second component reflects the rate of a chemical reaction.

The third component of the conductivity increase showed features quite different from the first and second ones. Its relative contribution to the total conductivity increase showed poor reproducibility but increased in all experiments with increasing temperature; its activation energy was much lower and its kinetics not well defined. Presently we have no satisfactory explanation for this component.

Chemical mechanism

The question arises as to which chemical mechanism is responsible for the strand break formation of the DNA radicals. In several publications the so-called C-4′ mechanism was proposed [4–7]. This mechanism was derived earlier based on the structure and the yield of altered sugar moieties isolated from irradiated DNA. Kinetic results obtained with aliphatic model compounds of small molecular weight [6, 28–30] showed that the rate constant of the phosphoric acid ester cleavage of 4′ type radicals increased with proton concentration between pH 7 and 2 by a factor of ~ 10^3. The activation energies were found to be between 11 – 14 kcal · mol^{-1}. The comparison with experimental data obtained with ssDNA shows that the activation energy found for strand break formation (13 kcal mol^{-1}) lies within this range. Similarly, the rate of strand break formation for ssDNA increases with proton concentration. The ratio of the rate constants of $k(\mathrm{H^+DNA})$ (pH = 4.3) and $k(\mathrm{DNA})$ at pH > 8.5 is 400 (Fig. 7). For poly U a corresponding increase by a factor of 550 was found [9].

These results point to the C-4′ mechanism as being responsible for the first part of the conductivity increase. In the experiments in which no third component in the time range of seconds was observed, the fast part accounted for 75% of the total conductivity increase, and hence 75% of the strand breaks may occur via the 4′ radical as an intermediate. This estimate is in agreement with earlier conclusions that the contribution of the C-4′ mechanism is not smaller than 50% [3].

Whether the 4′ radicals are formed only by reaction of OH with the sugar moiety or partly by an intermediate base radical is an open question. Recent results with poly U showed formation of strand breaks mediated by base radicals [8].

A mechanism for the second and third contributions cannot be derived from our present results. However findings obtained recently with 1,1-dimethoxy-3-bromo-propanyl-radicals as a model [30] support the suggestion that other radicals of the sugar moiety (*e.g.* 1′, 3′ or 5′ radicals) may also produce strand breaks but with slower rates as compared to that of the 4′ radical.

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