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Effect of γ-irradiation on the conformation of the native DNA molecule

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Abstract

Extensive investigation of γ-irradiated DNA solutions with the application of several informative physical methods suggested that at doses of 10–30 Gy the observed change in the size of the DNA molecule is due to a decrease in long-range interactions in the macromolecule. The comparison of the results of investigations of non-irradiated and irradiated DNA and its complexes with low molecular weight ligands over a wide range of ionic strengths showed that these interactions are electrostatic in nature and are due to a decrease in the charge density on the DNA molecule when its solutions are irradiated. In the irradiation dose range discussed here, the persistent length of the DNA molecule determined by short-range interactions in the chain does not undergo pronounced changes. It is shown that the free ligand in the irradiated solution can protect the DNA molecule against radiation damage. In contrast, the ligand bonded by intercalation does not exhibit this ability.

Keywords: Flow birefringence; Viscometry; Irradiation; Radiation damage; Conformation of DNA; Optical anisotropy

1. Introduction

When the action of ionizing radiation on the living cell is considered, the problem of the radiation damage of the DNA molecule is of greatest interest because it is this damage that plays the predominant role in the process of biochemical reactions which lead to cell destruction [1]. It should be pointed out, however, that the investigations of structural changes in the DNA molecule undergoing irradiation inside the cell are difficult because several biochemical processes occur in it. The information about the direct effect of ionizing radiation on the molecular structure of DNA is usually obtained in the study of irradiated dilute aqueous salt DNA solutions.

According to modern concepts, the radiation damage of the DNA molecule that occurs during the γ-irradiation of dilute solutions is due to the interaction of this molecule with the products of water radiolysis [2]. The character and degree of the damage depend on the radiation dose.

Many papers deal with the investigation of conformational changes which are manifested as a decrease in the effective volume of the DNA molecule. These changes are determined by the methods of molecular hydrodynamics and are usually explained by either a decrease in the persistent length of the DNA molecule as a result of single-strand breaks, or by a decrease in molecular weight caused by double strand breaks, or else by the formation of network structures [3–5]. It should be noted, however, that double-strand breaks in the DNA molecule appear at relatively high irradiation doses. This is evidenced

by the experimental results obtained in the investigation of irradiated DNA preparations isolated from different sources [6–8]. The minimum doses corresponding to the appearance of double-strand breaks obtained by different methods described in the above mentioned papers differ greatly. Thus, the results of the investigation of DNA PM-2 by neutral and alkaline electrophoresis in agarose gel have led to the conclusion that double-strand breaks are absent up to 200 Gy [6]. A direct study of the molecular weight of irradiated DNA by small-angle laser scattering has shown that double-strand breaks appear at a dose slightly exceeding 40 Gy [8]. Hence, up to 40 Gy the appearance of double-strand breaks in the DNA molecule is extremely unlikely.

The effect of conditions under which γ-irradiation is carried out (pH and ionic strength μ of the solution and the presence of oxygen) on the radiation damage of the DNA molecule has been discussed in the literature [7,9–11]. Some authors have explained the increase in the radiation effect with decreasing μ by an increase in the size of the target and, hence, in its accessibility for the attacking particles [11]. Other authors have supposed that the lower accessibility of the DNA molecule for the attack of active particles at high ionic strengths is due to its transition into another form [12]. The problem of the role played by oxygen in the process of radiation damage of the DNA molecule is difficult to solve. Oxygen can be bonded to the radicals of the macromolecule, thus increasing its damage but at the same time oxygen is an interceptor of hydrated electrons and hydrogen radicals, which leads to the weakening of the irradiation effect on the molecular structure of DNA [10].

The investigations of the behavior of low molecular weight ligands in the process of γ-irradiation of DNA solutions and their ability to protect the DNA molecule against radiation damage have been widely described in the literature. The indirect character of radiation damage of the DNA molecule in a dilute solution suggests that its protection is the result of the interaction of low molecular weight protectors with the products of water radiolysis and the subsequent inactivation of the products. The authors of refs. [13–15] suppose that any substance which is able to transform the molecules of water or a target into the inactive state can serve as a protector. It should be pointed out, however, that experiment indicated a marked difference in the efficiency of different ligands [16,17]. Using a number of low molecular weight substances, the authors of ref. [18] have observed partial protection of the DNA molecule even at doses above 200 Gy. In this case the protector concentration attained 10^{-3}–10^{-2} *M*. Other concepts of the protection mechanism of the DNA molecule against radiation damage also exist in the literature. Thus, it is assumed in ref. [19] that protection can be obtained if electron deficiency in the macromolecule is compensated for by the addition of electron donors. An adsorption mechanism of protecting the DNA molecule against radiation damage has also been proposed. In that mechanism the protector, which contains S–S bonds, plays the role of a joint localized on the DNA molecule. In this case the macromolecule loses its ability to undergo conformational changes [20].

The use of antitumoral antibiotics in the radiotherapy of tumors determines a special interest in the behavior of these ligands during the γ-irradiation of the cell. These ligands can increase or decrease the radiation damage of the cell [21,22]. More definite information about the role of these ligands could probably be obtained in the investigation of irradiated DNA solutions which contain antitumoral antibiotics. DNA solutions irradiated in the presence of various antibiotics have been investigated by the sedimentation method [23]. The molecular weight of native and denatured DNA was determined before and after γ-irradiation at doses of 0–500 Gy. The authors [23] could not find any correlation between the type of binding the antibiotics to DNA and their protecting or sensitizing properties. The conclusion that the free quinacrine and of that bonded to the DNA molecule play identical roles in protection against radiation damage [24] is not sufficiently convincing. Note that these authors ascribe equal efficiency to the intercalated ligand and to that bonded externally to the double helix of the DNA molecule.

In the present work combined investigations of

γ-irradiated and non-irradiated (native) DNA were carried out over a wide range of ionic strengths. The role of ligands capable and incapable of being bonded to the DNA molecule during γ-irradiation of solutions of DNA ligands was also studied.

2. Materials and methods

The calf thymus sodium salt of DNA (Serva Company) was used. The molecular weight MW was determined with the aid of a ratio relating it to intrinsic viscosity $[\eta]$ which was measured in a 0,15 M NaCl solution [25]. DNA concentration in solution was determined from its absorption at $\lambda = 270$ and $\lambda = 290$ nm after hydrolysis with $HClO_4$ [26]. The degree of nativeness of the investigated samples was determined from the value of the molar extinction coefficient E_{260}(P).

The main methods of investigation were viscometry, flow birefringence, and spectrophotometry.

Viscometry. Relative viscosities η_r of solutions with different DNA concentrations c depending on the flow rate gradient g were measured in a modified magnetic rotational Zimm and Crothers viscometer [27,28]. The intrinsic viscosity of DNA

$$[\eta] = \lim_{\substack{c\to 0 \\ g\to 0}} \frac{\eta_r - 1}{c}$$

was determined for all systems under investigation. According to the well-known Flory equation, $[\eta]$ is related to the parameters of the macromolecule by the equation

$$[\eta] = \Phi \frac{(\overline{h_0^2})^{3/2}}{M}\alpha^3 = \Phi \frac{(LA)^{3/2}}{M}\alpha^3 \qquad (1)$$

where $(\overline{h_0^2})^{1/2}$ is the mean-square end-to-end distance of the unperturbed macromolecule, α is the coefficient of its linear swelling, L and A are the hydrodynamic length and the length of the statistical chain segment, and Φ is the Flory coefficient.

Note that the value of $[\eta]$ proportional to the specific volume of the macromolecule is determined by both short- and long-range interactions in the chain (swelling and equilibrium rigidity). Hence, it is not possible to determine the variation of one of these parameters from a change in $[\eta]$. Only in the case of an ideal system ($\alpha = 1$, $\Phi = \Phi_0$) can eq. (1) be used for the determination of A.

2.1 Flow birefringence FB

FB was studied on an optical instrument with a semishaded elliptical compensator [29]. The dependence of the value of *FB*, Δn, on g was measured in solutions with different DNA concentrations, which allowed us to determine the dynamooptical constant

$$[n] = \lim_{\substack{c\to 0 \\ g\to 0}} \frac{(\Delta n/g)}{c\eta_0},$$

where η_0 is the solvent viscosity.

It is known that regardless of model concepts in the absence of form effects the $[n]/[\eta]$ ratio is proportional to the $(\gamma_1 - \gamma_2)$ value where γ_1 and γ_2 are the main polarizabilities of the macromolecule [30]. The value of $(\gamma_1 - \gamma_2)$ is determined mainly by intrinsic optical anisotropy proportional to the difference between the polarizabilities of its statistical segment $(\alpha_1 - \alpha_2) = S(a_{\parallel} - a_{\perp})$ [31].

Here S is the number of monomers, nucleotide pairs, in a segment, and $(a_{\parallel} - a_{\perp})$ is the difference in monomer polarizabilities along the axis of the double helix of DNA and that normal to it. The macromolecule may also be characterized by anisotropy caused by the effects of micro- and macroform [32–27].

Numerous experiments have demonstrated that in solutions of native DNA the macroform effect is negligible [38,39]. This follows from the equality of the values of $[n]/[\eta]$ and $(\Delta n/g)_{g\to 0}/\eta_0(\eta_r - 1)$ obtained for an infinitely dilute solution and for solutions of finite concentration [40,41]. Under these conditions, the ratio

$$\frac{[n]}{[\eta]} = \frac{[n]_i}{[\eta]} + \frac{[n]_{fs}}{[\eta]}$$

being measured depends on the intrinsic optical anisotropy and the anisotropy of the microform. Hence, the following equation may be written:

$$\frac{[n]}{[\eta]} = \frac{4\pi(n_s^2+2)^2}{45n_s kT}\cdot(a_\parallel - a_\perp)S + \frac{(n_s+2)^2}{180\pi\rho RT}\cdot\frac{(n_k^2-n_s^2)^2}{n_s^3}\cdot(L_2-L_1)M_g S \quad (2)$$

where n_k and n_s are the refractive indices of the polymer and the solvent, respectively, ρ is the polymer density, M_g is the molecular weight of the monomer, T is the absolute temperature, and $L_2 - L_1$ is the form factor which acquires a constant value at an asymmetry which is much less than that of the segment of the DNA molecule. Consequently, the $[n]/[\eta]$ ratio depends on the S value which characterizes the equilibrium chain rigidity and the values of $(a_\parallel - a_\perp)$. For native DNA we have $S = A/3 \cdot 4 = 2a/3.4$, where a is the persistent length of the macromolecule.

It can be seen that in solutions of native DNA $[n]/[\eta]$ does not depend on volume effects and is determined only by short-range interactions in the polymer chain.

2.2 Spectrophotometry

Spectrophotometric investigations were carried out on Specord UV-VIS spectrophotometer (Germany) and SF-26 spectrophotometer (Russia) and circular dichroism was measured on a Marc IV dichrograph (France).

DNA solutions were irradiated on a LMB-γ-1 unit (Russia) with a cesium source at a dose rate of 17 Gy/min. Ferrosulfate method was used for dosimetry.

3. Results and discussion

3.1 Irradiation of aqueous-salt DNA solutions

The intrinsic viscosity $[\eta]$ and the dynamooptical constant $[n]$ for the non irradiated DNA and for that irradiated with doses of 10–15 Gy in a 0.1 M NaCl solution were determined. Experiments showed that irradiation changes markedly the $[n]$ and $[\eta]$ values (Fig. 1) but has no appreciable effect on the $[n]/[\eta]$ ratio at an irradiation dose of even more than 30 Gy (Fig. 2). This fact implies that at these doses the secondary structure and equilibrium rigidity of the DNA molecule, which are determined by short-range interactions in the chain, do not undergo any marked changes. The coincidence of the circular dichroism spectra of irradiated and non-irradiated DNA solutions also indicates that irradiation with doses of 10–30 Gy has no effect on the double-helix structure. No appreciable differences are also observed in the E_{260}(P) values for these systems (Fig. 3).

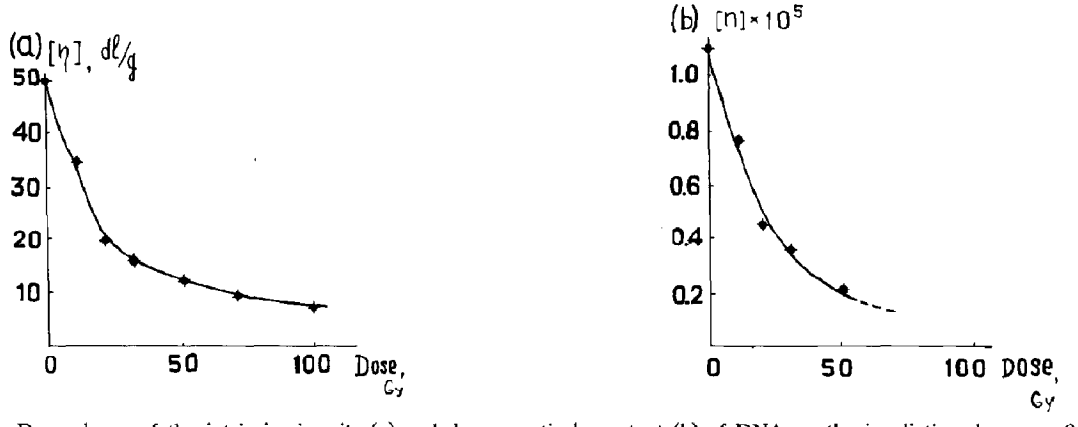

Fig. 1. Dependence of the intrinsic viscosity (a) and dynamooptical constant (b) of DNA on the irradiation dose; $\mu = 0.1$ M, MW = $8 \cdot 10^6$ daltons.

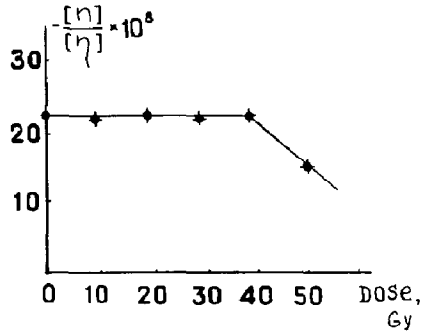

Fig. 2. Dependence of $[n]/[\eta]$ on the irradiation dose; $\mu = 0.1$ M, MW = $8 \cdot 10^6$.

These experimental results suggest that the observed change in the hydrodynamic volume of the DNA molecule caused by γ-irradiation of its solutions results from a change in long-range interactions in the macromolecule. We will now attempt to consider the nature of these interactions.

3.2 Investigation of DNA solutions containing low molecular weight ligands

As already mentioned, the distortion of the molecular structure of DNA that results form the γ-irradiation of its solutions proceeds from the interaction between DNA and active particles, the products of water radiolysis. Hence, it might be suggested that in the case of their inactivation the radiation damage of the DNA molecule can be avoided. This suggestion is confirmed by the results of comparative investigations of non-

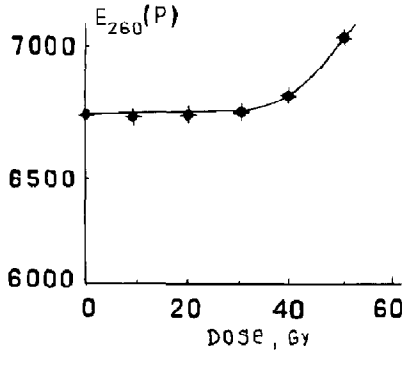

Fig. 3. Dependence E(P) for DNA solution on the irradiation dose; $\mu = 0.1$ M, MW = $8 \cdot 10^6$.

irradiated and irradiated by dose of 10 Gy DNA solutions which contain low molecular weight ligands.

Caffein and 8-bromocaffeine which virtually are not bonded to DNA were chosen as ligands. This absence of bonding is indicated by the coincidence of absorption spectra of the mixture and the sum of the spectra of its components and by invariable concentration dependence $(\eta_r - 1)/c$ of DNA solutions when the ligand content in the solvent used for dilution was varied. It should be borne in mind that in the case of a ligand that can be bonded to DNA, for correct concentration investigations the ligand concentration in the solvent should coincide with that of the free ligand in the initial solution. Note that the absorption maxima of these ligands and DNA are too close to carry out spectrophotometric titration.

The investigations of DNA solutions with different caffeine contents showed that the presence of this ligand in the irradiated solution protects DNA against radiation damage. This protection increases with ligand concentration c in solution. This is indicated by the dependence of the $[n]$ and $[\eta]$ values of irradiated DNA on c. It was shown that at $c = 0.7$ molecules of the ligands per pair of nucleotides, the values of $[n]$ and $[\eta]$ for DNA irradiated with a dose of 10 Gy coincide with those obtained for nonirradiated DNA. In this case the number of caffeine molecules in solution greatly exceeds that of active particles that appear at a dose of 10 Gy. Just like in the absence of the ligand in solution, irradiation does not affect the $[n]/[\eta]$ ratio.

More detailed investigations were carried out for the DNA–8-bromocaffeine system. The results of viscometric investigations are shown in Fig. 4 which presents the dependence of $[\eta]$ on 8-bromocaffeine concentration at different irradiation doses. However, the ligand concentration required for attaining the $[\eta]$ value of the nonirradiated DNA depends on the radiation dose. Experiment showed that in the range of 10 to 30 Gy the $[\eta]$ value of irradiated DNA attained that for non-irradiated DNA. Above 30 Gy the $[\eta]$ value of irradiated DNA remains lower than that of non-irradiated DNA at all c values tested. Under these conditions, irreversible changes

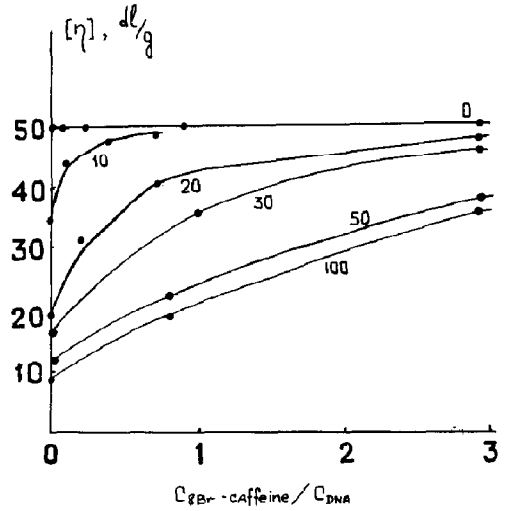

Fig. 4. Dependence of $[\eta]$ or DNA on the concentration of 8-Br-caffeine in solution at different irradiation doses: $\mu = 0.1$ M; MW = $8 \cdot 10^6$; $c_{DNA} = 0.1$ g/L.

probably occur in the molecular structure of DNA. This is also indicated by a decrease in the $[n]/[\eta]$ ratio.

The experimental results suggest that a free ligand in the irradiated solution can protect the DNA molecule against radiation damage.

We believe that the use of ligands that can be bonded to DNA may provide additional information about the mechanism of DNA protection against radiation damage. Acriflavine was used as such a ligand because the relationships of its complexation to DNA at different ionic strengths have been investigated in detail in ref. [40]. The results of this work indicate that in 0.1 M NaCl solution acriflavine is bonded to the DNA molecule by intercalation. With increasing concentration of bonded acriflavine, the $[n]/[\eta]$ ratio increases in absolute value, which shows that the persistent length of the macromolecule also increases. This fact in combination with the increase in the length of the DNA molecule also determines an increase in intrinsic viscosity which exhibits a linear dependence on the amount of the ligand bonded to DNA.

Comparative investigations of non-irradiated and irradiated DNA complexes with different acriflavine contents were carried out in a 0.1 M NaCl solution. Acriflavine concentration in solution was determined from its extinction coefficient $E_{452} = 46\,800$. The data on spectrophotometric titration were used to determine the number of acriflavine molecules bonded to DNA per pair of nucleotides r and the content of free ligand in solution m. The complete coincidence between the dependences of r on m obtained for non-irradiated and irradiated DNA and acriflavine (Fig. 5) indicates that irradiation with a dose of 10 Gy has no effect on the degree of bonding of acriflavine to DNA at $\mu = 0{,}1$. Figure 6 shows the dependence of $[\eta]$ on r for irradiated and non-irradiated complexes. The plots demonstrate that the change in the hydrodynamic behavior of the complex due to γ-irradiation does not depend on the ligand concentration in the complex and coincides with the change observed for free DNA. This result suggests that acriflavine bonded to DNA, at least by intercalation, does not protect it against radiation damage.

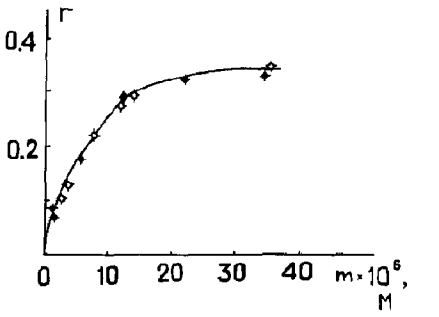

Fig. 5. Curve of of acriflavine bonding with DNA: (○) non-irradiated components, (●) irradiated components; $\mu = 0.1$ M.

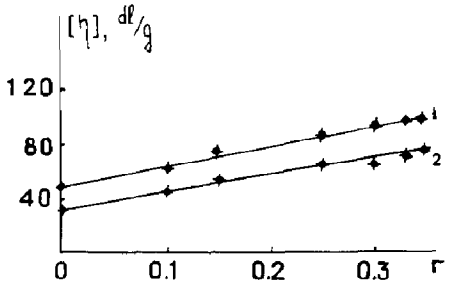

Fig. 6. Dependence of intrinsic viscosity on the degree of binding: 1 – for non-irradiated DNA + acriflavine complex, 2 – for DNA + acriflavine complexes irradiated at a dose of 10 Gy; $\mu = 0.1$ M.

A free ligand the concentration of which depends on r and the ionic strength of the solution is known to be present in the DNA–ligand equilibrium system. In the DNA–acriflavine systems under investigation, the effect of the free ligand on the results of the γ-irradiation of DNA was not observed. Note that this cannot be explained by the low activity of acriflavine molecules because the rate constant for their interaction with the products of water radiolysis even slightly exceeds those obtained for caffeine and 8-bromocaffeine [16]. The comparison of the concentration of free acriflavine with that of active particles of various natures, which appear at a given irradiation dose showed that even at a maximum r value the content of free acriflavine in solution is insufficient for the manifestation of protective properties.

In this connection systems which contain acriflavine in excess were investigated. The results show that when a certain concentration of the free ligand is attained, the value of $[\eta]$ for the irradiated DNA becomes equal to that obtained for nonirradiated DNA (Fig. 7). This result confirms the conclusion that a free ligand can protect the DNA molecule against radiation damage. Of considerable interest is the problem of the role of a ligand externally bonded to DNA in the process of γ-irradiation of its solutions. To elucidate this problem, the effect of γ-irradiation on the hydrodynamic behavior of complexes under ionic conditions ($\mu = 0.005$ M) under which acriflavine is

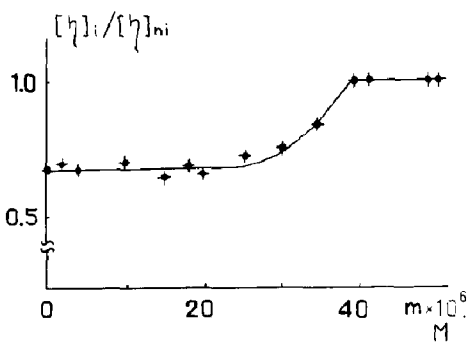

Fig. 7. Dependence of the ratio of intrinsic viscosities of the DNA + acriflavine complex irradiated at dose 10 Gy and nonirradiated on the concentration of free acriflavine in the solution; $\mu = 0.1$ M.

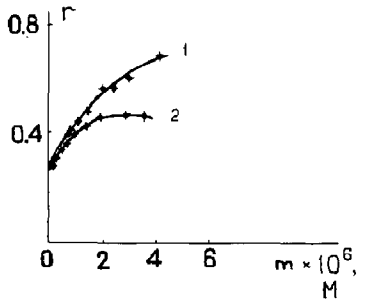

Fig. 8. Curves of bonding of acriflavine with DNA at $\mu = 0.005$ M; 1 – non-irradiated components, 2 – irradiated at dose of 10 Gy.

bonded to DNA not only by intercalation but also outside the double helix was investigated. In this case this bonding type is due to electrostatic interactions between the charges of the ligand and of DNA. Since under these conditions acriflavin is bonded to DNA in at least two ways, the spectrophotometric titration of the ligand by DNA solutions does not provide correct information about the concentrations in solution of the free ligand and that bonded to DNA. Hence, we used the method of fluorescent titration that has been developed for this system previously [42]. The dependence of r on m obtained in this case for nonirradiated DNA and the ligand is shown in Fig. 8, curve 1.

Let us examine the results of viscometric investigations carried out at $\mu = 0.005$. The increase in $[\eta]$ of the nonirradiated DNA caused by the intercalation of the ligand proceeds more slowly than at $\mu = 0.1$ because r is determined not only by the intercalated but also by the externally attached ligand. At a concentration of the bonded ligand that corresponds to the completion of the intercalation process, an increase in r leads to a certain decrease in $[\eta]$, which is probably due to the screening of the phosphate groups by the externally bonded ligand (Fig. 9, curve 1). A similar result has been obtained in ref. [42].

After the irradiation of this system by a dose of 10 Gy, the dependence of $[\eta]$ on r acquired a linear character similar to that observed in a 0.1 M NaCl solution (Fig. 9, curve 2). The resemblance of the hydrodynamic behavior of complexes irradiated at high and low ionic strengths

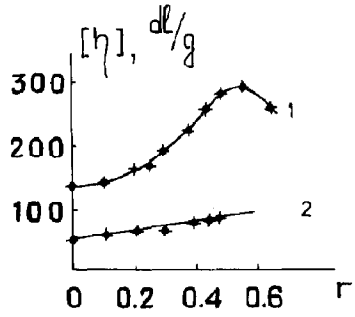

Fig. 9. Dependence of intrinsic viscosity of the DNA+ acriflavine complex on the degree of bonding at $\mu = 0.005$ M; 1 – non-irradiated, 2 – irradiated at a dose of 10 Gy.

suggested that after irradiation only the intercalation way of bonding of acriflavine to DNA exists. This situation can take place only if irradiation leads to a break of the bond between DNA and the ligand attached externally by a double bond.

This suggestion was confirmed by the results of fluorescent titration of irradiated DNA and acriflavine in a 0.005 M NaCl solution. The maximum value of r (Fig. 8, curve 2) coincided with the found for nonirradiated and irradiated DNA and acriflavine at $\mu = 0.1$ M, i.e. under the conditions when bonding occurs only by intercalation (Fig. 5, curve 2). A similar result was obtained in the spectrophotometric titration of irradiated components. The appearance of the isobestic point on absorption plots shows that only one bonding type exists.

All experimental data show that under the ionic conditions chosen by us ($\mu = 0.005$ M) irradiation breaks the bonds between the DNA molecule and the ligand, which are due to electrostatic interactions. Consequently, the effect of irradiation on the molecular parameters of DNA is similar to some extent to that of increasing ionic strength of solution.

These results lead to the suggestion about the nature of the decrease in volume effects in the DNA molecules, caused by the γ-irradiation of its solutions. We believe that irradiation leads to a decrease in the charge density on the DNA molecule, thus causing a weakening of electrostatic interactions, both intramolecular interactions and those between the phosphates of the DNA molecule and the positively charged ligand [43]. This assumption explains the absence of the effect of irradiation with doses of 10–30 Gy on the $[n]/[\eta]$ ratio, which is related to short-range interactions in the macromolecule. In fact, it has been shown in refs. [44–49] that in the range of ionic strengths $\mu > 0.003$ M, the change in the hydrodynamic volume of the DNA molecule upon the variation in ionic strength is due only to a change in long-range electrostatic interactions. The role of short-range electrostatic interactions in the DNA chain is manifested in the range of $\mu < 0.003$ M. In this range the decrease in ionic strength leads to a drastic increase in $[n]/[\eta]$ because of an increase in the persistent length of the macromolecule. Moreover, the linear character of the dependence of $[\eta]$ on $\mu^{-1/2}$ is no longer obeyed. Hence, it should be expected that at ionic strength $\mu < 0.003$ M irradiation should lead to a less pronounced increase in $[n]/[\eta]$ (again because of the increase in the charge density on the DNA molecule). Therefore, the concept of the decisive role played by single-strand breaks in the observed decrease in the hydrodynamic volume of the DNA molecule at doses of 10–30 Gy seems to us incorrect. We do not deny the possibility of the existence of single-strand breaks but we only assert that in the range of irradiation doses considered here they do not affect the equilibrium rigidity of the molecule of native DNA. It is noteworthy that the authors of ref. [50] have obtaned a controlled number of single-strand breaks by treating native DNA with pancreatie DNase. It was shown that five breaks in a statistical segment do not lead to marked changes in the persistence length of the DNA molecule.

We think that the validity of the above suggestion about the nature of radiation damage of the DNA molecule at doses 10–30 Gy is also confirmed by the results of the investigation of nonirradiated and irradiated DNA solutions at different ionic strengths. Here we restricted ourselves to tue range of ionic strengths that affect only long-range electrostatic interactions. The dependence of $[\eta]$ on $\mu^{-1/2}$ (Fig. 10, curve 1) is linear and agrees with that repeatedly obtained previously in this range of ionic strengths. In the case of DNA irradiated with a dose of 10 Gy at

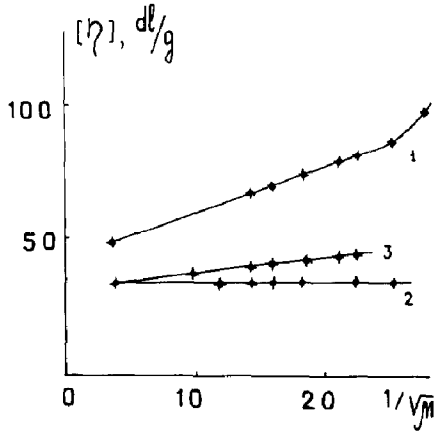

Fig. 10. Dependence of the intrinsic viscosity of DNA on the ionic strength of solution μ; 1 – non-irradiated, 2 – irradiated at a dose 10 Gy at different ionic strengths, 3 – irradiated at a dose of 10 Gy at $\mu = 0.1$ *M*.

different ionic strengths, equal values of $[\eta]$ were obtained. They are tentatively shown as the dependence of $[\eta]$ on $\mu^{-1/2}$ (Fig. 10, curve 2). It appears that the molecule of irradiated DNA behaves similarly to the uncharged macromolecule. However, this result can be explained by the fact that with decreasing ionic strength of the irradiated solution the charge density on the DNA molecule changes greatly and this leads to the apparent absence of the dependence of $[\eta]$ on μ. This is confirmed by the following additional experiments: DNA irradiated in a 0.1 *M* NaCl solution was transformed by dialysis into solutions with lower ionic strengths. The values of $[\eta]$ obtained at these μ values had a linear dependence on $\mu^{-1/2}$. However, as was to be expected, this dependence was much weaker than that for the non-irradiated DNA (Fig. 10, curve 3).

We think that all these experimental data confirm the assumption on the nature of the radiation damage of the DNA molecule during γ-irradiation of its solutions by doses of 10–30 Gy. Nevertheless, the answer to the question of the mechanism of changes in charge density on the DNA molecule is not unequivocal. It might be assumed that irradiation facilitates the accessibility of the DNA molecule for free cations and ensures their stronger bonding than in the absence of irradiation. It should be pointed out, however, that this problem requires further investigations.

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