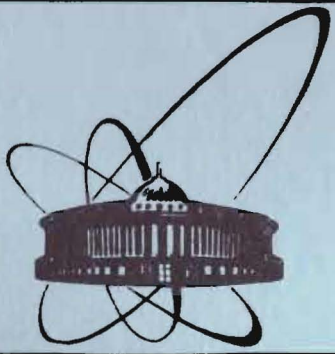

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G.Erzgräber*, I.L.Lapidus

RELATION BETWEEN SEDIMENTATION BEHAVIOUR OF DNA-MEMBRANE COMPLEXES AND DNA SINGLE- AND DOUBLE-STRAND BREAKS AFTER IRRADIATION WITH γ-RAYS, PULSE NEUTRONS AND ^{12}C IONS

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INTRODUCTION

The results of a number of experimental investigations point out that DNA in nuclei of mammalian cells is organized as supercoiled subunits [1-5].

A simple and convenient method to study the size of these subunits is to determine changes in sedimentation properties of the so-called DNA-membrane complexes (DMC), which sediment in the neutral sucrise density gradient with ethydium bromide as an easily identified aggregate with the velocity corresponding to the irradiation dose (in case of the seldom-ionizing radiation) [6,7].

It is assumed that presence of DNA single-strand breaks (SSB) leads to despiralization of supercoiled subunits, which results in a decrease of the whole aggregate sedimentation velocity. If our assumption is true, the rejoining of SSB during the process of postirradiation incubation will show itself as DMC repair. To confirm this hypothesis is one of the purposes of our investigation.

For better argumentation of the idea about existence of compact subunits and about mechanisms of their destruction by irradiation we carried out comparative experiments with different types of radiation and extended our investigations to the area of large doses.

This paper is a sum of results obtained in the investigation of mammalian cells (V79-4) and human peripheral blood lymphocytes affected by γ-rays (^{137}Cs), pulse neutrons and accelerated ^{12}C ions. On the basis of these data we suggest a new method to determine the yield of DNA breaks.

MATERIALS AND METHODS

Lymphocites. Fresh donor blood was mixed with the 0.9% NaCl solution in proportion 1:1 layed on the top of the mixture of 5.6% Ficoll solution and Visotrast (17:3) with a density of 1.077±0.001 g/ml. After centrifugation for 30 min at a temperature of 20° C with a rate of 4000 rpm the lymphocytes were separated from other blood components in the form of a white ring. They were washed in cold PBS-glucose by double centrifugation (10 min, 1000 rpm, 20°C). Then cells were resuspended in Eagle MEM medium with 20% human blood serum and kept in closed tube in 5% CO_2 atmosphere. Such short-living cultures of lymphocytes having 10^6 cells per ml with less than 3% of erythrocytes keep a good viability for 7 days.

V79-4 cells. V79-4 Chinese hamster cells were cultivated by standard monolayer technique and grown in the Eagle MEM medium with 15% serum and antibiotics.

Before irradiation the exponentially growing cultures were removed by trypsinization and a 10^6 cells/ml suspension was made of them. For reduction of possible damages due to trypsinization the cell suspension was incubated for 45 min at 37° C and then was kept at 0-4° C till irradiation.

Irradiation. γ-irradiation was carried out at the "Svet" (^{137}Cs) facility with the 4.66 Gy/min dose rate; ^{12}C ion irradiation was performed at the cyclotron U-200 at the Laboratory of Nuclear Reactions (JINR, Dubna). This has been described in detail in [8]. For neutron irradiation the fast neutron pulse reactor IBR-2 in the JINR Neutron Physics Laboratory (Dubna) was used [9]. Cells were irradiated at 0-4°C and then kept at this temperature untill further treatment.

Lysis of cells, sucrose gradients, centrifuging conditions. Lysis solution: 1% SDS, 0.2% deoxycholate-Na, 0.05 M Na-cytrate, 0.05 M EDTA, pH 7-7.5. Sucrose gradient: 4.6 ml 5-20% sucrose in 1 M NACL, 0.01 M Tris-HCl, 0.01 M EDTA, 6 μg/ml ethydium bromide, pH 7-7.5. 0.2 ml of the lysis solution was layered on the top of the sucrose gradient and then 0.1 ml of suspension with 10^5 cells layered for lysis. The duration of lysis was 1 hr. The duration of centrifugation was 1 hr at 30000 rpm at 20°C in the swing-out rotor of the centrifuge "VAC-602".

Measurement of DMC position. Ethydium bromide, which is present in the sucrose gradient, is intercalated between DNA bases. When sedimenting in the form of the reticular aggregate, DNA as a component of DMC is enriched with ethydium bromide, and this one can find the position of DMC with the fluorescence method (the excitation light is 360 nm, the emission light is 590 nm).

RESULTS AND DISCUSSION

Figure 1 shows the results of our investigation of repair of damaged DNA from Chinese hamster cells (V79-4) (fig.1a) and from human lymphocytes (fig.1b) depending on the γ-irradiation doses for different time of postirradiation incubation of cells at 37° C. The lower curve describes the relative sedimentation ve-

locity (RSV) immediately after the γ-irradiation; other curves were obtained after incubation during 10, 30 and 60 min respectively.

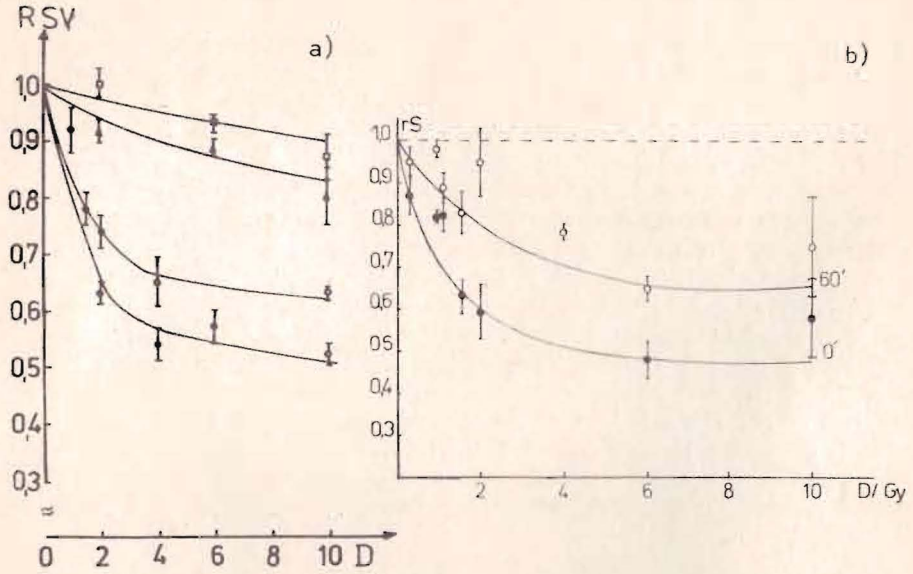

Fig.1. Dependence of the relative sedimentation velocity upon the γ-radiation dose for different postirradiation incubation periods. Abscissae - doses in Gy; ordinates - the relative sedimentation velocity. a - Chinese hamster cells V79-4 (• - without incubation, o - 10 min incubation, Δ - 30 min incubation, □ - 60 min incubation); b - Lymphocytes (• - without incubation, o - 60 min incubation).

To compare the DMC repair kinetics with the kinetics of DNA SSB rejoining, obtained by the traditional method of DNA sedimentation in alkaline sucrose gradient, we determine the probability of "DMC survival" from the initial slope of the rS(D) curve, it is equal to:

$$1 - rS^* = e^{-M_0 sD}, \qquad (1)$$

where D is the dose, s is the yield of DNA SSB, M_0 is the molecular weight of one subunit, rS* is the percentage of DMC, despiralized due to DNA SSB.

Since for $D = D_{37}$ $M_0 sD = 1$, one can determine (without data on M_0) the relative SSB yield in this way:

$$s_t/s_0 = D_{37,0}/D_{37,t}, \qquad (2)$$

where indices t and O mean the duration of incubation or its absence. Points in Fig.2 are the results of processing the data from Fig.1 in the above way. The solid line is the SSB-rejoining kinetic curve, obtained by Körner et al. [10] for Chinese hamster cells of the same line by means of the traditional sedimentation technique. From this figure one can see that SSB rejoining and DMC repair depend on the incubation time in the same way. Noteworthy is the considerable percentage of unrepaired SSB in lymphocytes after 1-hour postirradiation incubation. These data agree with the well-known fact of high radiosensitivity of lymphocytes [11,12] and confirm the assumption that their higher sensitivity to γ-radiation is due to their poor ability of rejoining DNA breaks.

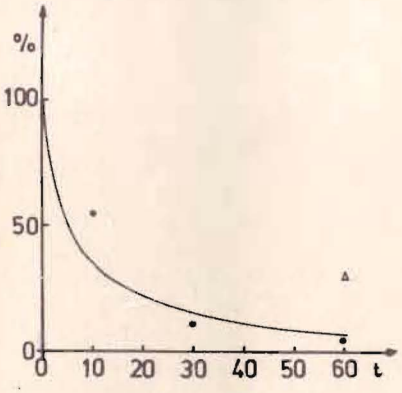

Fig.2. Kinetics of DNA-membrane complex repair. The solid line shows the kinetics of SSB rejoining according to (Körner et al.(1978)); points show our data (• are the cells of the Chinese hamster V79-4, Δ are lymphocytes). Abscissae - incubation time in min; ordinates - the relative numbers of SSB in %.

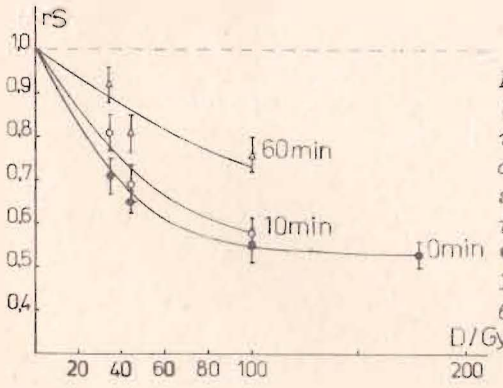

Fig.3. Dependence of the DMC (V79-4) RSV upon the ^{12}C-ions dose for different incubation periods. Abscissae - dose in Gy; ordinates - RSV in relative units. • - Without incubation, o - 10 min incubation, Δ - 60 min incubation.

The results of investigation of DMC repair after irradiation of cells by accelerated ^{12}C ions are presented in Fig.3. Comparing Figs.1 and 3, one can see that for the letter case the velocity of DMC repair is less than that at γ-irradiation.

It is known that an average yield of DNA SSB decreases as LET grows [13]. Moreover in this case the spatial distribution will be more nonuniform. If at the analysis of DMC sedimentation one would proceed from the assumption about existence of the independent supercoiled subunits with the much smoller molecular weight than that of the whole genome, then the curve for the radiation of higher LET must be higher than it was expected taking into account decrease of the DNA SSB yield. Fig.4 present the results of the comparative experiments with γ-rays, neutrons and ^{12}C ions for Chinese hamster cells (Fig.4a) and lymphocytes (Fig.4b). Though the DNA SSB yield for ^{12}C is approximately half as large as that for γ-rays, the rS(D) curve runs higher than it is expected on the basis of this fact. In our opinion, the explanation of this phenomenon is as follows. After γ-irradiation the DNA SSB distribution is in good agreement with the Poisson distribution, i.e., in each supercoil subunit DNA SSB arise with equiprobability. Heavier particles with high LET cause DNA SSB only in the subunits where the "hit events" took place. Thus, after neutron or ^{12}C ion irradiation at equal (low) doses the number of subunits, where the "hit" occured, is less, than after γ-irradiation.

There is one more reason for such sedimentation behaviour of DMC affected by heavy particles; a yield of DNA double-strand breaks (DSB) in this case is considerably larger than the one after γ-irradiation. For example, the DSB-to-SSB yields ratio is approximately 0.01 in the case of γ-irradiation and 0.1 in the case of ^{12}C ions. The influence of DNA DSB on the DMC sedimentation properties almost was not studied. To solve this problem the character of rS(D) dependence was investigated in the γ-ray dose range over 50 Gy, where an average of one DNA DSB per a subunit was expected. The results of these investigations are presented in Fig.5. After RSV decrease in the dose range down to 50 Gy its value increases again as the dose grows up to 550 Gy, and the narrow maximum with rS ~1.5 is formed. Then RSV again decreases down to the value obtained at the dose ~50 Gy. To make the explanation of these unexpected data more clear, one more curve is plotted in Fig.5. It shows the part of DNA released from DMC due to DSB as a function of the dose - F(D). The solid curve was calculated theoretically [2], triangles show our data obtained on the cells V79-4 [14]. The rS(D) curve can be explained in the following way: in the dose range up to 50 Gy the behaviour of the curve is the result of the despiralization of DNA supercoil subunits owing to SSB, which leads to a slowing sedimentation. At the dose of the order of 50 Gy all subunits are despiralized and complexes have the slowest sedimentation velocity. The part of released DNA at 50 Gy amount only to 3%, therefore complexes destructed in this way (and free fragments of DNA) do not affected the value of rS. In the next dose range from 50 to 550 Gy the relative velocity of sedimentation grows quickly and the part of released DNA achieved 67%. We assume that as a result of release of DNA fragments a considerable compactization of complexes takes place, and their sedimentation velocity grows. After the maximum at 550 Gy the RSV again decreases, since the complexes velocity of free DNA fragments which decreases due to decrease in the molecular weight. Besides, destruction of the remaining complexes goes on, which leads to the rupture of their compactness.

But all these considerations are of some interest only for the case when all DNA-containing components sedimentate in one aggregate with a certain mean velocity. This assumption is proved by our experiments aimed at studying the sedimentation behaviour of the mixture, which consists of nonirradiated, or

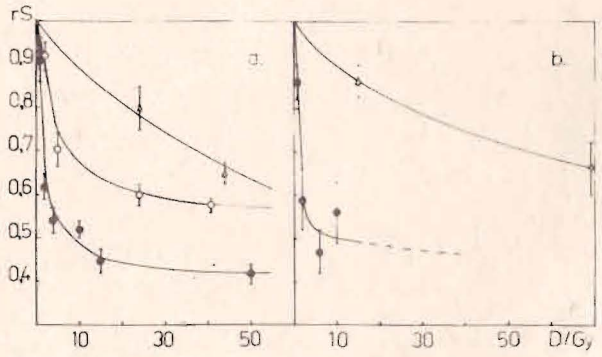

Fig.4. Dependence of the DMC RSV upon the γ-radiation dose (•) neutron dose (o) and ^{12}C-ions dose (Δ); a - V79-4; b - human lymphocytes.

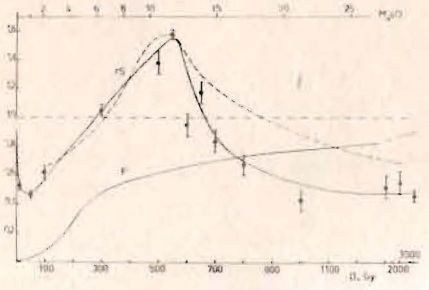

Fig.5. Dependence of the DMC RSV and percentage of free DNA upon the γ-radiation dose. The dashed curve is calculated by means of Eq.(3). Abscissae - below - doses in Gy, above - the mean number of DSB per subunit. Ordinates - the DMC RSV and percentage of free DNA in relative units.

checking, cells (30%) and cells irradiated with a high dose (70%). The results listed in the Table show, that if the mixture includes cells irradiated with dose less than 550 Gy, the sedimentation velocity of the aggregate decreases (because of non-destroyed complexes), and if there are cells irradiated with doses over 550 Gy, the velocity of sedimentation increases.

Table

Relative sedimentation velocity of mixture of nonirradiated (30%) and irradiated (70%) cells

Dose, Gy	rS_{dose}	rS_{mix}
300	1.17	1.05
500	1.50	1.13
750	0.60	0.97
800	0.60	1.07
1000	0.43	0.93

On the basis of the afore-mentioned facts one can calculate the aggregate sedimentation velocity in the first approximation by the RSV of the DNA-containing mixture components in the following way:

$$rS = A\,rS_{desp.} + B\,rS_{d.s.} + F\,rS_{fragm.} \quad (3)$$

where A is the percentage of subunits having a relative sedimentation velocity $rS_{desp.}$ and despiralized due to SSB (having no DSB); B is the percentage of destroyed subunits which lost DNA fragments due to DSB, their sedimentation velocity being $rS_{d.s.}$; F is the percentage of free DNA with the sedimentation velocity $rS_{fragm.}$.

On the basis of obvious assumptions that at the 550 Gy dose A = 0 (practically all subunits have DSB) and the mean value of $rS_{fragm.}$ is 0.3 we obtain $rS_{d.s.}$ = 3.8. The results of these calculations are shown in Fig.5 by the dashed curve with the maximum at 550 Gy. In fact, $rS_{fragm.}$ (and consequently $rS_{d.s.}$) depends upon the irradiation dose; nevertheless, one can affirm that our assumption on the DSB role in the growth of the curve in the 50-550 Gy dose range is true.

Our ideas were also checked in comparative experiments with other types of radiation. The dependence rS(D), obtained for γ-rays, neutrons and ^{12}C ions is presented in Fig.6. It follows

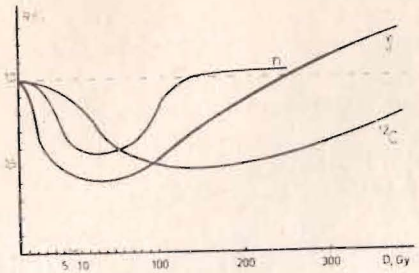

Fig.6. Relative sedimentation velocity of DMC vs dose of γ-rays, neutrons and ^{12}C ions. Abscissae - doses in Gy; ordinates - DMC RSV in relative units.

from the data of papers [2,6] that, for example, α-particles with LET 66 keV/μm are more effective in the DNA release process, than γ-rays, and ^{12}C ions release the DNA percentage, approximately comparable with the one for γ-rays. However, here the variation of the spatial distribution of DNA breaks manifests itself again. A shape of the curves in Fig.6 clearly demonstrates the DNA DSB influence on the DMC sedimentation behaviour: for the neutrons (in the LET range from 30 to 1000 keV/μm) an increase of rS(D) starts at lower doses and for ^{12}C ions at higher doses, than in the case of γ-rays.

The results presented in this paper reveal the possibility of using the DMC study method for the DNA breaks yield estimation. The same curve rS(D) helps in principle to determine at the same time the SSN yield (at low doses) and the SSB yield (at high doses) in a wide dose range. The calculation of the DNA DSB yield at the γ-irradiation will be published.

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REFERENCES

1. Cook P.L., Brazell J.A. J.Cell.Sci., 1976, 22, p.287-302.
2. Regel K., Günter K., Kampf G. Environm.Biophys., 1983, 21, p.175-200.
3. Hartwig M. Acta biol.med.germ., 1978, 37, p.421-432.
4. Filippovich I.V. et al. Int.J.Radiat.Biology, 1982, 42, 1, p.31-44.
5. Fender H. Sedimentationsuntersuchungen an chromosomaler Säuger-DNS. Dissertation, AdW der DDR, 1979.
6. Абель Х., Эрцгребер Г. Радиобиология, 1983, 23, 1, с. 14-20.
7. Абель Х., Эрцгребер Г., Лангрок К. Радиобиология, 1983, 23, 4, с.435-438.

8. Абель Х. и др. Радиобиология, 1984, 24, 3, с,300-304.
9. Лапидус И.Л., Назаров В.М., Эрцгребер Г. ОИЯИ, Р19-84-286, Дубна, 1984.
10. Körner I.J., Günter K., Malz W. studia biophysica, 1978, 70, 3, p.175-182.
11. Kutlaca I.J. et al. Mut.Res., 1982, 94, p.125-131.
12. Jonnsson L., Carlsson J., Nilsson K. Int.J.Radiat.Biol., 1982, 41, 4, p.411-420.
13. Günter K., Schulz W. Biophysical Theory of Radiation Action. Academie Verlag, Berlin, 1983.
14. Эрцгребер Г., Лапидус И.Л., Айхорн. ОИЯИ, 19-83-491, Дубна. 1983.

Received by Publishing Department
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Эрцгребер Г., Лапидус И.Л. E19-85-385

Взаимосвязь между седиментационными свойствами ДНК-мембранных комплексов и одно- и двунитевыми разрывами ДНК при облучении γ-лучами, импульсными нейтронами и ионами ^{12}C

Представлены экспериментальные данные о седиментационном поведении ДНК из клеток китайского хомячка V79-4 в результате облучения клеток γ-лучами (^{137}Cs), импульсными нейтронами /реактор ИБР-2 Лаборатории нейтронной физики ОИЯИ, Дубна/ и ускоренными ионами ^{12}C /циклотрон У-200 Лаборатории ядерных реакций ОИЯИ, Дубна/ в широком диапазоне доз. С помощью экспериментов с излучениями разного качества обоснованы предположения о роли одно- и двунитевых разрывов ДНК в изменении седиментационных свойств ДНК-мембранных комплексов. Обсуждается возможность оценки индукции и репарации разрывов ДНК на основе кривой зависимости относительной скорости седиментации комплексов от дозы облучения.

Работа выполнена в Лаборатории ядерных проблем и Лаборатории нейтронной физики ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна 1985

Erzgräber G., Lapidus I.L. E19-85-385

Relation between Sedimentation Behaviour of DNA-Membrane Complexes and DNA Singe- and Double-strend Breaks after Irradiation with γ-Rays, Pulse Neutrons, and ^{12}C Ions

The experimental data on sedimentation behaviour of DNA-membrane complexes at irradiation of the Chinese hamster cells (V79-4) in a wide dose range of ^{127}Cs γ-rays, pulse neutrons (reactor IBR-2, Laboratory of Neutron Physics, JINR, Dubna) and accelerated ^{12}C ions (cyclotron U-200, Laboratory of Nuclear Reactions, JINR, Dubna) are presented. An assumption on the role of DNA single- and double-strend breaks in changing the sedimentation properties of DNA-membrane complexes has been confirmed by the experiments with radiation of different quality. The possibility of estimating induction and repair of DNA breaks on the basis of dependence of the relative sedimentation velocity of complexes on the irradiation does is discussed.

The investigation has been performed at the Laboratory of Nuclear Problems and Laboratory of Neutron Physics, JINR.

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