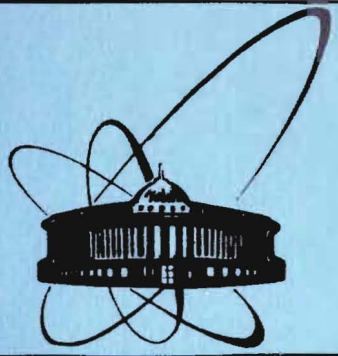

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SEDIMENTATION PROPERTIES OF DNA-MEMBRANE COMPLEXES AND YIELD OF DNA BREAKS AT IRRADIATION OF MAMMALIAN CELLS

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I. INTRODUCTION

A series of investigations has revealed an existence of supercoiled DNA subunits, connected with the nuclear membrane. They are called DNA membrane complexes (DMC) and their molecular weight M_o is approximately $3.34 \cdot 10^{-15}$ g /1-5/. Sedimentation behaviour of these complexes has been shown to change considerably after irradiation of cells /4/. This phenomenon can be explained through an assumption that single-strand breaks (SSB) of DNA lead to despiralization of DMC /5/. When studying the sedimentation behaviour of DMC in cells of the Chinese hamster after mixing the corresponding fractions of irradiated and non-irradiated DMC, the authors of paper /6/ have shown that the position of the DMC peak after centrifuging is the superposition of contributions of individual DMC.

The relative sedimentation velocity (RSV) r_s decreases very quickly after irradiation of cells with doses of the order of several Greys from 1 (the reference material is the non-irradiated cells) down to approximately 0.4 ($D \geq 10$ Gy) /4,5/. For different types of cells the initial parts of relations $r_s(D)$ do not coincide, which can be due to different sizes of DMC in some types of cells /4,5/. The RSV difference from the control level observed at the given irradiation dose reduces during the postirradiation incubation practically to nil. The time-dependence curve coincides with kinetics of DNA SSB reparation, determined by the sedimentation method /4/.

When irradiation doses are sufficiently high (of the order of 100 Gy), the value of r_s for DMC of the Chinese hamster V79-4 cells increases again, has a local maximum at $D \sim 550$ Gy, and reaches the value 0.4 again /4/. It should be noted that irradiation with large doses (and, consequently, a large number of double-strand breaks (DSB) of DNA) leads to the release of DNA fragments from DMC /8/. Since irradiation can induce DNA damages of another type (not only SSB) which can change the DMC sedimentation behaviour in the opposite way, as compared to SSB, this can be a possible reason for the local maximum. Most probably, these damages are DSB of DNA.

It is very important to develop new methods for the determination of the DNA DSB yield, and we have made an attempt to analyse the relation between the dependence

$r_S(D)$ and induction of DNA breaks. On the basis of experimental data, assumptions on quantitative relations are put forward and most probable hypotheses are discussed.

2. INDUCTION AND REPARATION OF DNA SSB

Due a discrete absorption of energy of ionizing irradiation, the induction of DNA SSB is an incidental process. The presence of DNA SSB in DMC leads to a reduction of RSV to some value r_S^+ (see Fig. 1). In view of the Poisson distribution of energy absorption at γ-irradiation, the fraction f of DMC "survived" after irradiation with the dose D (i.e. without DNA SSB) is

$$f = e^{-M_o s_\perp D}, \qquad (1)$$

where M_o is the molecular weight of DMC, $s_\perp$ is the DNA SSB yield per a dose unit and a gramm. Since the position of the peak is the superposition of individual contributions of separate DMC, the final sedimentation velocity as a function of the dose is

$$r_S(D) = e^{-M_o s_\perp D} + r_S^+ (1 - e^{-M_o s_\perp D}). \qquad (2)$$

This function describes well the initial part of the curve $r_S(D)$. The plateau region allows one to determine the value r_S^+ and the inclination of the straight line in $-\ln(r_S - r_S^+)/(1-r_S^+)$ vs D plot equals $M_o.s_\perp$. A more accurate estimate can be obtained through the χ^2 minimization.

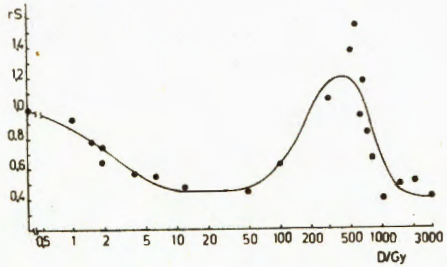

Fig. 1. The relative sedimentation velocity of DNA - membrane complexes vs the dose of γ-irradiation. Abscissae : doses in Grey (logarithmic scale); ordinates : DMC RSV in relative units.

If the DNA SSB reparation is an incident process, it can be presented by an exponential function. Therefore, one can write :

$$S(t) = S_r e^{-\lambda t} + S_{ir}, \qquad (3)$$

where s_r is the fraction of repairable SSB, s_{ir} is the fraction of irrepairable SSB, λ is the decay constant. Introducing the fraction of irrepairable breaks as $f = s_{ir}/s_\perp$ we may rewrite eq. 3 in the form :

$$S(t) = S_\perp \left[(1-f)e^{-\lambda t} + f\right] \qquad (4)$$

substituting eq. 4 in eq. 2, we obtain the dose-time dependence r_S:

$$r_S(D,t) = e^{-M_o s_\perp \left[(1-f)e^{-\lambda t}+f\right] D} + r_S^+ (1 - e^{-M_o s_\perp \left[(1-f)e^{-\lambda t}+f\right] D}) \qquad (5)$$

For the V79-4 cells the parameters $M_o s_\perp$, r_S^+, λ, and f have the following values : $M_o.s_\perp = 0.534$, $r_S^+ = 0.525$, $\lambda = 0.14$ hour^{-1}, $f = 0.062$. The optimal values are determined by means of the programme MINUIT (CERN). The optimal value of the sum $q^2 = \sum_{i=1}^{n} \frac{(r_{si,theor} - r_{si,exper})^2}{\sigma_i^2}$ is 61.4 (σ_i^2 are the deviations) for the number of degrees of freedom n = 15, which suggests that the actual deviations of experimental data are greater than the statistical errors (σ_i). According to eq. 5 the given effect r_S can be observed at various combinations of D and t values. The $r_S(D,t)$ dependence is shown in Fig. 2.

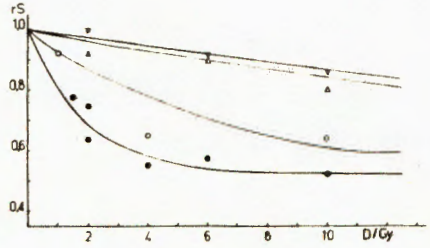

Fig. 2. The relative sedimentation velocity of DNA - membrane complexes vs the dose of γ-irradiation for several times of incubation allowing the reparation of DNA SSB. Abscissae : doses in Grey; ordinates : DMC RSV in relative units.

3. FUNCTION $r_S(D)$ IN THE REGION OF HIGH IRRADIATION DOSES

The most interesting part of the curve $r_S(D)$ is the region of the local maximum at irradiation doses over 100 Gy (see Fig. 1). The most probable is the assumption that this behaviour of the curve is due to the changes in the sedimentation behaviour of DMC as a result of DNA DSB. However, the relation between the yield of DNA DSB and the behaviour of the $r_S(D)$ curve is quite complicated. If we consider the quantity r_S as the superposition of contributions of separate DMC (r_{Si}), i.e.

$$r_S = \frac{1}{N}\sum_{i=1}^{N} r_{Si} , \qquad (6)$$

where N is the number of complexes, we may discuss some hypotheses concerning the mentioned relation.

Eq. 6 can be rewritten in the form:

$$r_S = f_0 + f_1 r_S^+ + \sum_{i=1}^{\infty} f_{2j} r_{S2j} , \qquad (7)$$

where f_0 is the fraction of DMC without breaks (RSV of these DMC is taken as 1); f_1 is the fraction of DMC only with DNA SSB; f_{2j} are the fractions with j - DNA DSB; r_{S2j} is the contribution of the corresponding DMC with j - DNA DSB to r_S. The first two terms of expression 7 describe the initial part of the $r_S(D)$ curve. Fractions f_0, f_1, f_{2j} can be easily calculated under the assumption on the Poisson distribution of DNA SSB and DSB over DMC at γ-irradiation:

$$f_0 = e^{-M_0(S_1+S_2)D} , \qquad (8a)$$

$$f_1 = (1 - e^{M_0 s_1 D})e^{-M_0 s_2 D} , \qquad (8b)$$

$$f_{2j} = e^{-M_0 s_2 D}\,\frac{(M_0 s_2 D)^j}{j!} . \qquad (8c)$$

Parameters $M_0 s_1$ and $M_0 s_2$ are the numbers of DNA SSB and DSB, respectively, per a complex and a dose unit. The only unknown parameter r_{S2j} cannot be calculated, as the structure of damaged complexes is very complicated and actually unknown, but the main characteristic features of these DMC will be discussed below.

4. SEDIMENTATION PROPERTIES OF DMC WITH DSB

The presence of DNA DSB in DMC can obviously change their properties. The first DSB does not, however cause the fragmentation of DMC and, therefore, we can assume that the sedimentation properties of DMC are not changed. Large fragments arising from DMC with several DSB (2,3,...) lead to the increase of the RSV. On the other hand, great number of DNA DSB cause full fragmentation of DMC, which is concerned with decreased RSV of DNA agregate again.

Two DSB per DMC are critical as a new state of DMC arises. A part of DNA is bound to the membrane and the remaining part is released. At the same time additional DSB cause only a gradual decrease of the molecular weight of DNA fragments accompanied by diminishing RSV of DNA-containing agregate.

The simplest distribution of r_{S2j} reflecting the decreased situation is the following:

$$r_{S21} = r_S^+$$

$$r_{S22} = r_{max}$$

$$r_{S2j} = r_{Sfrag} = 0.4 \text{ for } j > 2 \quad /2/.$$

Then the optimal values of the parameters are $M_0 s_1 = 0.29$ (0.16 - 0.52), Gy^{-1} $M_0 s_2 = 0.0049$ (0.0035 - 0.0072) Gy^{-1}, $r_S^+ = 0.39$, $r_{max} = 3.5$. The errors are determined by means of MINUIT, MINOS command. If DMC with several DSB /2,3,4/ have increased RSV, the obtained production of DSB slightly increases ($1.8 - 2.1 \cdot 10^{12}$) $Gy^{-1}g^{-1}$, the value of q^2, however, increases.

5. CONCLUSION

The character of the function $r_S(D)$ can be explained on the basis of analysis of the relation between yields of DNA SSB and DSB and changes in sedimentation properties of DMC. The available experimental data are in good agreement with the hypothesis that already the first SSB of DNA leads to a considerable reduction of the RSV, while its increase after that requires 2 or more DSB of DNA. The proposed approach is highly productive, because it allows to calculate both quantities on the basis of the same curve obtained, naturally, under identical experimental conditions: the initial part of the curve provides information on occurring SSB of DNA, and the position of the local maximum allows one to calculate the yield of DNA DSB.

The corresponding values for yields of DNA SSB and DSB obtained by the method proposed by us are : $s_1 = 0.9.10^{14}$ $Gy^{-1}g^{-1}$, which corresponds to the value given in the literature /9/ $s_1 = 1.2.10^{14}$ $Gy^{-1}g^{-1}$; $s_2 = 1.5.10^{12}$ $Gy^{-1}g^{-1}$, which is comparable with the value that we obtained by the usual sedimentation method - $s_2 = 3.10^{12}$ $Gy^{-1}g^{-1}$. It is easy to estimate the reparation decay constant, for the V79-4 cells we obtained $\lambda = 0.14$.

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Эрцгребер Г., Козубек С., Лапидус И.Л. E19-85-626

Седиментационные свойства ДНК-мембранных комплексов и выход разрывов ДНК при облучении клеток млекопитающих

На примере клеток китайского хомячка проводится анализ зависимостей относительной скорости седиментации ДНК-мембранных комплексов от дозы облучения и длительности пострадиационной инкубации. Предполагается, что начальная часть рассматриваемой кривой содержит информацию об образовании однонитевых разрывов ДНК, а положение локального максимума позволяет рассчитать выход двунитевых разрывов ДНК. Приводится оценка величины константы распада.

Работа выполнена в Лаборатории ядерных проблем ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна 1985

Erzgräber G., Kozubek S., Lapidus I.L. E19-85-626

Sedimentation Properties of DNA-Membrane Complexes and Yield of DNA Breaks at Irradiation of Mammalian Cells

The dependence of the relative sedimentation velocity of DNA-membrane complexes on the dose of irradiation and time of incubation of Chinese Hamster cells is analysed. It is concluded that the initial part of the curve provides the information on the occurrence of single strand breaks in DNA; the position of the local maximum allows us to calculate the yield of DNA double strand breaks. The reparation decay constant can be estimated as well.

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

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