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G.Erzgräber, I.L.Lapidus, H.Abel

INDUCTION AND REPAIR OF DAMAGES OF CHROMATINE SUPERCOILED SUBUNITS AFTER γ-IRRADIATION

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1. INTRODUCTION

Existence of compact subunits associated with the nuclear membrane in the interphase nuclei of the mammalian cells was shown by different authors[1-4].

It is supposed that after γ-irradiation of the cells the supercoiled structure of DNA-membrane complexes (DMC) is destroyed due to single-strand breaks (SSB)[5,6], that is expressed in decrease of sedimentation velocity as compared with the complexes from the nonirradiated (control) cells. If SSB are the actual reason for such sedimentation behaviour, there is a possibility of investigating the kinetics of SSB repair with a relatively simple method (sedimentation in sucrose density gradient with ethidium bromide in dose range up to 10 Gy without radioactive labelling).

By researching the sedimentation behaviour of DMC and using fractional irradiation, one can judge about the degree of SSB repair. The purpose of this paper was the investigation of induction and repair of SSB by the DMC-sedimentation method. For the first time we applied this method in a wide dose range (up to 3000 Gy) and made an attempt to estimate also the yield of DNA double strand breaks.

2. MATERIAL AND METHODS

The growth condition for the Chinese hamster cells (V79-4), the isolation of lymphocytes of human peripheral blood and the neutral sucrose gradient sedimentation technique were described earlier[6]. Concentration of ethidium bromide was 30 μg/ml in all experiments. The samples were irradiated at the "SVET" facility (^{137}Cs) at the temperature 0°- 4°C. Postirradiation incubation continued for 10, 30 or 60 min at 37°C.

3. RESULTS AND DISCUSSION

Figure 1a shows experimental values of the relative sedimentation velocity of DMC of V79-4 cells in dependence upon the γ-irradiation dose immediately after the irradiation and after 10, 30, 60 min of postirradiation incubation. On the basis of paper[6] we calculated the values of rS*, the so-called survival

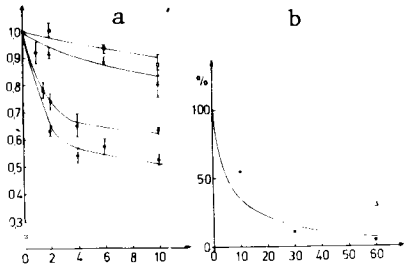

Fig.1a. Dependence of the relative sedimentation velocity upon the γ - radiation dose for different incubation periods. • - without incubation; o - 10 min incubation; Δ - 30 min incubation; □ - 60 min incubation. Abscissae: doses in Gy; ordinates: the relative sedimentation velocity. Fig.1b. Kinetics of the DNA-membrane complex repair. The solid line shows the kinetics of SSB rejoining according to Kerner et al.[7]; 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 %.

of supercoil DMC, and further D_{37} was determined. From the equation $M_0 \cdot D_{37} \cdot \lambda = \text{const}$, where M_0 is the molecular weight of one subunit and λ is the yield of SSB per Gy and Dalton (λ_0 - immediately after the irradiation, λ_t - as a result of postirradiation incubation during t) one can determine the relative yield of SSB without information about M_0:

$$\lambda_t / \lambda_0 = D_{37,0} / D_{37,t}.$$

Figure 1b shows the dependence of SSB relative yields upon the duration of postirradiation incubation. The solid line is the SSB-rejoining kinetic curve obtained by Kerner et al.[7] for the Chinese hamster cells of the same line by means of a usual sedimentation technique; the dots show our data. One can see from the figure that SSB rejoining and DMC repair are in the same dependence upon the incubation time. That means that the complex repair kinetic allows one to judge about induction and repair of SSB. We would like to note that there are too many unrepaired SSB in lymphocyte cells after 1 hour of postirradiation incubation. These data agree with the well-known fact of high radiosensitivity of lymphocytes[8,9] and confirm the assumption, that their higher sensitivity to γ -radiation is due to their poor ability of repairing DNA breaks. Experiments of Kerner et al. allowed one to determine the kinetic of SSB rejoining exposed to γ -radiation doses of about 100 Gy. By using our method it seems possible to study the kinetics of SSB rejoining in the dose range up to 10 Gy. Figure 2 shows the experimental results of the investigations of the complex repair in the dose range up to 100 Gy. The results allow the conclusion, that at γ -irradiation doses up to 100 Gy the repair of complexes and, consequently, of SSB have a constant velocity within 60 minutes of postirradiation incubation.

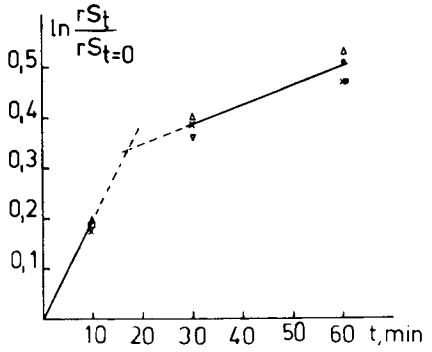

Fig.2. Repair velocity of the damaged DNA-membrane complexes. Abscissae: postirradiation incubation time in min; ordinates: logarithm of the relative velocity $\ln \frac{rS_t}{rS_{t=0}}$ after incubation during the time. This velocity is normalized to the one at t = 0.

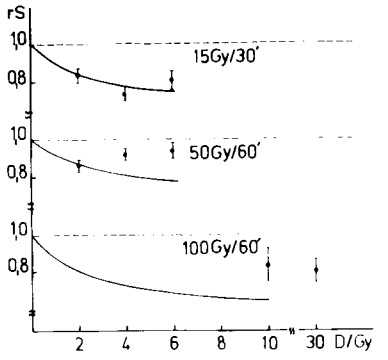

Fig.3. Experimental results of fractionated irradiation. Abscissae: the second irradiation dose in Gy; ordinates: the relative sedimentation velocity normalized to the one after the first dose irradiation and the corresponding incubation at 37°C.

The method of fractionated irradiation allows one to check if the SSB repair gives rise to supercoiled complexes with the same sedimentation behaviour, as it follows from the above data with allowance for the breaks, which appear after the second irradiation. In our experiments we used following regime: 15 Gy + 30 min incubation + 2 or 4 or 6 Gy + 60 min incubation + 2 or 4 or 6 Gy; 100 Gy + 60 min incubation + 10 or 30 Gy. The obtained results are presented in fig.3. The complex sedimentation velocity after irradiation of the cells equals one. Solid lines show relative sedimentation velocities calculated on the basis of the curve (fig.1) illustrating the data obtained immediately after the irradiation. If our assumptions regarding the role of SSB in the sedimentation behaviours of DMC are correct, then after irradiation in the mentioned fractionation regime we should obtain a number of SSB, which is a sum of the SSB unrepaired during interfraction incubation and the SSB due to the second irradiation. The experimental values of the relative sedimentation velocity are shown by dots. They are in good agreement with the expected values for the following fractionation regime: 15 Gy + 30 min at 37°C + 2,4,6 Gy. That means, that SSB repair gives rise to the complexes, which will behave in accordance with the curves in fig.1 after the second irradiation. In the two other fractionation regimes the expected sedimentation

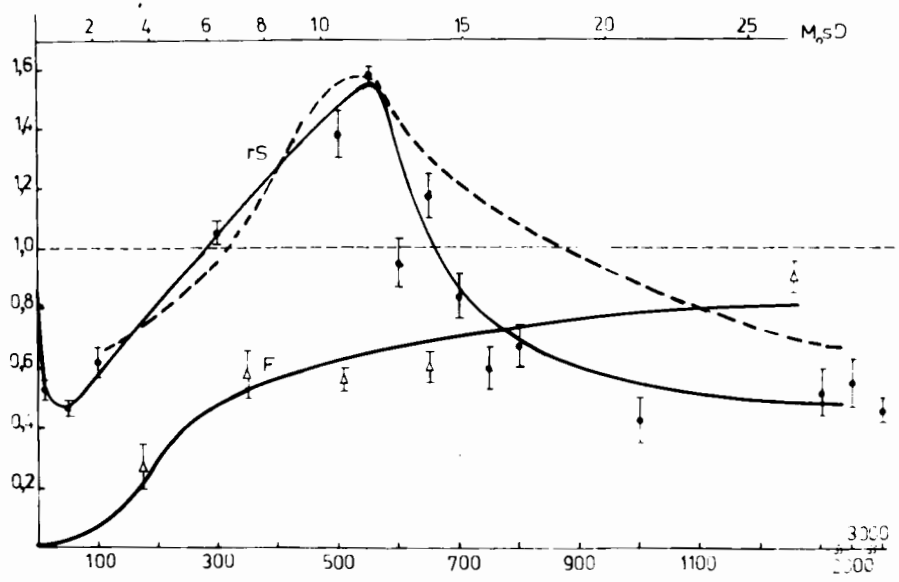

Fig.4. The relative sedimentation velocity of DMC and percentage of free DNA plotted against the γ-radiation doses. The dashed curve is calculated by means of Eq. (1). Δ - our data published in ref./14/. Abscissae: below: doses in Gy; above: the mean number of DSB per subunit (yield of DSB for γ-radiation is $4.5\ 10^{-12}\,Gy^{-1}\,D^{-1}$). Ordinates: the relative sedimentation velocity of DMC and percentage of free DNA in relative units.

behaviour was not observed. To examine this effect the DNA sedimentation was investigated in the dose range over 100 Gy. The results are seen in fig.4. To make the explanation of these unexpected data more clear, one more curve is plotted in fig.4; it shows the part of DNA F released from DMC due to DSB. The solid curve was calculated theoretically/10/; triangles show our experimental data obtained on the cells V79-4/11/. If one divides in mind the curve rS(D) in four parts, one can explain its behaviour in each dose range in the following way. In the 0-50 Gy range the behaviour of the curve is the result of despiralization of DNA supercoil subunits owing to SSB, which leads to a slower sedimentation. At the dose of the order of 50 Gy all subunits are despiralized and sedimentation of complexes has the slowest relative velocity. The part of released DNA at 50 Gy amounts only to 3%, therefore complexes destructed in this way (and free fragments of DNA) do not affect 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 achieves 67%. We assume, that as the 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 sedimentation relative velocity again decreases, since the complex velocity is more and more affected by the sedimentation velocity of free DNA fragments, which decreases due to decrease in the molecular weight. Besides, the 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 non-irradiated, or checking, cells (30%) and cells irradiated with a high dose (70%). The results listed in the table show, that if the mixture includes the cells irradiated with doses 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 non-irradiated (30%) and irradiated (70%) cells

Dose (Gy)	^{r}S dose	^{r}S 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 this basis of the afore-mentioned facts one can calculate the aggregate sedimentation velocity in the first approximation by the relative sedimentation velocity of the DNA-containing mixture components in the following way:

$$rS = A \cdot rS_{desp.} + B \cdot rS_{d.s.} + F \cdot rS_{fragm.}, \qquad (1)$$

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.4 by 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.

Thus, the results of our investigation proved, that the technique developed to measure superspiralization of DNA structure affected by radiation is suitable for estimation of relative yields of DNA single- and double-strand breaks in various dose ranges, but under absolutely identical conditions. Using the molecular weight of the subunit as a known value, one can find the numbers of SSB and expect estimation of the number of DSB. These calculation will be published.

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REFERENCES

1. Bauer W., Vinograd I.I. Mol.Biol., 1970, 47, p.419.
2. Cook P.R., Brazell I.A. Cell Biol., 1975, 19, p.261.
3. Cook P.R., Brazell I.A. Nature, 1976, 263, p.679.
4. Hartwig M. studia biophysica, 1977, 63, p.75.
5. Hartwig M. Acta Biol.Med.Chem., 1978, 37, p.421.
6. Абель Х., Эрцгребер Г., Лангрок К. ОИЯИ, 19-82-379, Дубна, 1982.
7. Körner I.J., Günther K., Malz W. studia biophysica, 1978, 70, p.175.
8. Johansson L., Carlsson J., Nilsson K. Int.J.Radiat.Biol., 1982, 41, p.411.
9. Kutlaca R. et al. Mut.Res., 1982, 94, p.125.
10. Vexler F.B. et al. Int.J.Radiat.Biol., 1982, 41, p.453.
11. Regel K., Günther K., Kampf G. Environm.Radiat.Biophys., 1983, 21, p.175.

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Эрцгребер Г., Лапидус И.Л., Абель Х. E19-83-493

Повреждение и восстановление суперспиральности структур хроматина при γ-облучении

Методом седиментации ДНК-мембранных комплексов изучали индукцию и репарацию однонитевых разрывов ДНК при γ-облучении клеток китайского хомячка линии V79-4. Впервые осуществлено применение этого метода при облучении клеток большими дозами γ-излучения; представлена кривая, характеризующая седиментационное поведение ДНК-мембранных комплексов из клеток, облученных в дозах от 0 до 3000 Гр. Высказывается предположение о роли двухнитевых разрывов ДНК в изменении относительной скорости седиментации комплексов при облучении клеток в дозах выше 50 Гр.

Работа выполнена в Лаборатории ядерных проблем ОИЯИ.

Сообщение Объединенного института ядерных исследований. Дубна 1983

Erzgräber G., Lapidus I.L., Abel H. E19-83-493

Induction and Repair of Damages of Chromatine Supercoiled Subunits after γ-Irradiation

The induction and repair of the DNA single-strand breaks during γ-irradiation of the Chinese hamster cells (V79-4) have been investigated using the method of the DNA-membrane complex sedimentation. For the first time this method has been employed for the case of high-dose γ-irradiation of cells; the curve is presented, which characterises the sedimentation behaviour of DNA-membrane complexes from cells irradiated with doses from 0 to 3000 Gy. An assumption is put forward concerning the role of DNA double-strand breaks in changing the relative sedimentation velocity of complexes during the irradiation of cells with doses over 50 Gy.

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

Communication of the Joint Institute for Nuclear Research. Dubna 1983