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DNA STRUCTURES AND RADIATION INJURY

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ABSTRACT

In the present paper experimental results from radiobiological investigations of the sedimentation behaviour of damaged and restored DNA-subunits attached to the nuclear membrane have been summarized.

The studies were carried out preferably with Chinese Hamster cells V79-4 irradiated with different kinds of radiation (gamma-rays, neutrons and carbon ions) using the nucleoid sedimentation technique.

Single-strand breaks relax the supercoiled DNA in the subunits resulting in a decreased sedimentation velocity. Rejoining leads to a correct restoration of the structure as can be studied by means of postincubation irradiation. Double-strand breaks release DNA fragments, again leading to an increased sedimentation velocity. If the average number of the induced double-strand breaks per subunit increases to a number higher than one, the measured results suggest that the structures should not be restored completely. The results are compatible with a new repair model developed in our laboratory on the assumption that, firstly, the single DNA subunits are the sensitive target rather than the whole DNA and, secondly, the repair of DNA damage takes place independently in each subunit.

INTRODUCTION

Today it is well established that the chromosomal DNA of a cell is the primary critical structure. However, we are not sure that it is the DNA as a whole which determines radiation sensitivity. There is certain evidence suggesting that the large DNA molecules of a mammalian cell are subdivided into certain segments or subunits /1,2/. In this context the question arises: Is it only the stabilization or the arrangement of the DNA molecule which requires such subdivision, or are there other important phenomena involved, too. One of the biological features which may be functionally combined with the subunits is the repair of DNA lesions. In the last few years we have carried out some radiobiological experiments and certain theoretical microdosimetric studies aimed at a more complete description of these structures and their biological meaning.

In the first part of the report we would like to summarize some experimental data which yield information about the existence and the properties of these structures. In the second part we present our first attempts to find quantitative relations between the radiation energy absorption event distribution in these structures, the induced DNA lesion patterns, and their repair.

DNA ORGANIZATION

In Figure 1 certain levels of our concept for DNA organization are represented schematically. We are mainly concerned with the third level.

The double-stranded helical DNA is wound around the nucleosome core forming a nucleosome chain which goes to make up the chromosome. According to the conclusions which are drawn from our studies of radiation-induced DNA release in mammalian cells /2,4/, we have to expect that this nucleosome chain is

subdivided into DNA structures by its attachment to the nuclear matrix. Our theoretical microdosimetric approach /2/ yields the following properties of these DNA subunits:

- All the chromosomal DNA of mammalian cells consists of loop like nuclear matrix-attached DNA superstructure units with a molecular weight (M_o) of about 10^9 g/mol.
- These DNA structures are rather compact structures. The diameter of their volume amounts to some tenths of a micrometer.

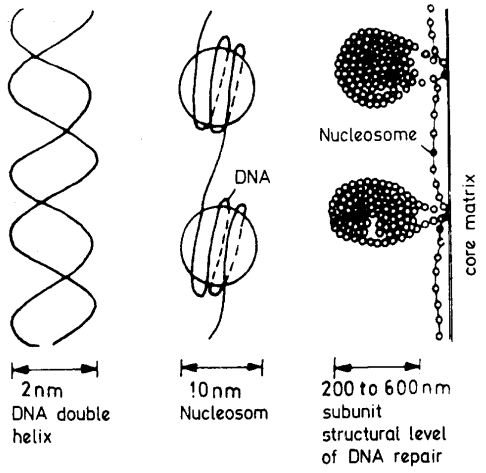

Fig. 1. A schematic representation of our concept for DNA organization in mammalian interphase cells (from /3/).

Our first hint of the existence of DNA substructures with such a molecular weight was obtained from the results for the determination of DNA double-strand breaks induced by different radiation types /4/. For the estimation of double-strand break rates, the reciprocal of the mean DNA molecular weight is plotted as a function of radiation dose as represented in Figure 2. The crucial point of these results was that the curves intersect the ordinate axis at a point which is characterized by a molecular weight of about 10^9 g/mol. This is significantly less than the average DNA molecular weight per chromosome.

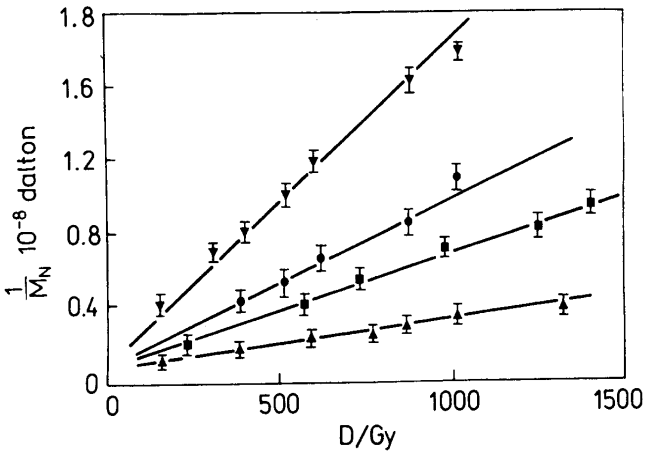

Fig. 2. Dose effect relationship between the reciprocal of the mean molecular weight of DNA released after irradiation of V79-4 cells with gamma-rays (▲), 6 MeV-neutrons (■) and alpha-particles 50 keV/µm (●) resp. 100 keV/µm (▼) (from /4/).

Of course the compactness of the DNA structures implies a very distinct DNA supercoiling.
Under defined conditions of cell lysis these supercoiled subunits form aggregates. Their sedimentation behaviour is influenced by both the DNA single-strand breaks (relaxation of DNA) /1,5,6,7,8/ and the double-strand breaks (release of DNA segments). Both these effects are assayed by the nucleoid sedimentation technique, with the correlation between double-strand breaks and the sedimentation behaviour of the aggregated nucleoids first being found by us /7/. Therefore both these effects are applied to the investigation of DNA lesions and their repair.

DNA INJURY AND REPAIR - PHENOMENOLOGICAL STUDIES

Radiation-induced DNA single-strand breaks lead to relaxation of supercoiled DNA in the substructures. This results in a decreased sedimentation velocity for the aggregated subunits after cell lysis. In Figure 3 the relative sedimentation velocity (rS) is exhibited in relation to the gamma dose for human lymphocytes, Chinese hamster cells V79-4 and mouse Ehrlich ascites tumor cells /8/.

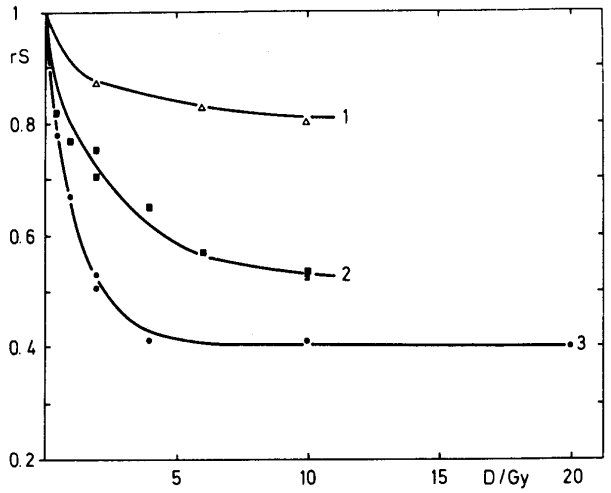

Fig. 3. Dependence of the relative sedimentation velocity (rS) of the aggregated subunits on the gamma-ray dose for different cell lines: 1-Mouse Ehrlich ascites tumor cells, 2-Chinese hamster cells (V79-4), 3-Human lymphocytes (from /8/).

Qualitatively, the sedimentation behaviour of the subunits is similar in different cells. The quantitative differences can be explained by the different molecular weights of DNA subunits for the various cell lines. In the dose range where rS is nearly independent of the dose, the DNA in all subunits are relaxed due to single-strand breaks.

Figure 4 shows the dependence of rS on the gamma-dose after different postirradiation incubation periods. After 60 min incubation times rS of the subunits is similar to those from undamaged cells. This means that approximately all single-strand breaks are rejoined.

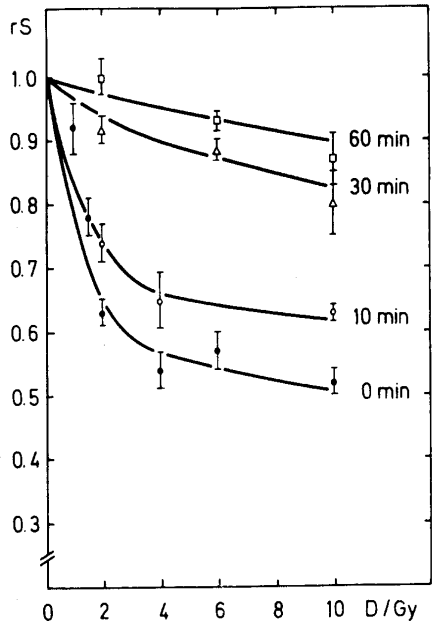

Fig. 4. Dependence of the relative sedimentation velocity (rS) of DNA subunits (V79-4 cells) on the gamma-ray dose for different incubation periods after irradiation: 14 experiments: (from /7/).

In order to investigate whether the rejoining of the breaks leads to undamaged DNA subunits, the following experiments were carried out. After a 30 minute postirradiation incubation period following irradiation at a dose of 15 Gy, the cells were again irradiated at different doses.

In Figure 5 it is shown that after a 30 minute incubation rS increases as expected. Following the second irradiation rS decreases with the dose exhibiting a sedimentation behaviour which looks like that after the first irradiation.

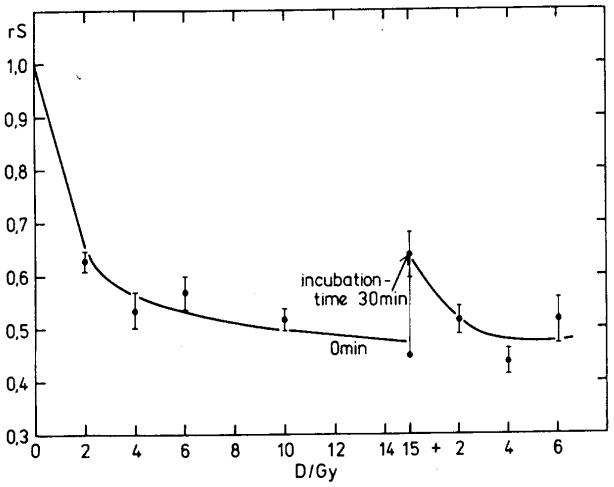

Fig. 5. Dependence of the relative sedimentation velocity (rS) of DNA subunits (V79-4 cells) on gamma-ray dose without incubation (up to 10 Gy, at the left) and with an incubation period of 30 minute after irradiation with 15 Gy followed by a second irradiation (at the right).

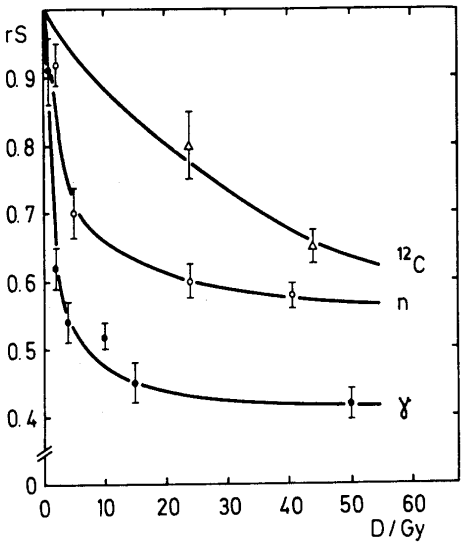

Fig. 6. Dependence of the relative sedimentation velocity (rS) of DNA subunits (V79-4 cells) on the dose of gamma-rays, neutrons and carbon ions. Number of experiments: 14, 4, 3, respectively (from /7/).

Whereas for gamma-rays, the break probabilities in all subunits were the same (Poissonian break distribution), high LET-radiations induce a more inhomogeneous one. Therefore, at low doses of high LET-radiations DNA strand breaks were induced only in some subunits of a cell, but often many breaks are induced in one subunit. Consequently, at similar doses the fraction of the damaged and, therefore, relaxed subunits, exhibited in rS is significantly lower in case of carbon ions than that after gamma-irradiation. Figure 6 shows a comparison of rS effects induced by gamma-rays, neutrons with the mean energy of 0.7 MeV and carbon ions with the LET-value of 250 keV/μm on Chinese hamster V79-4 cells.

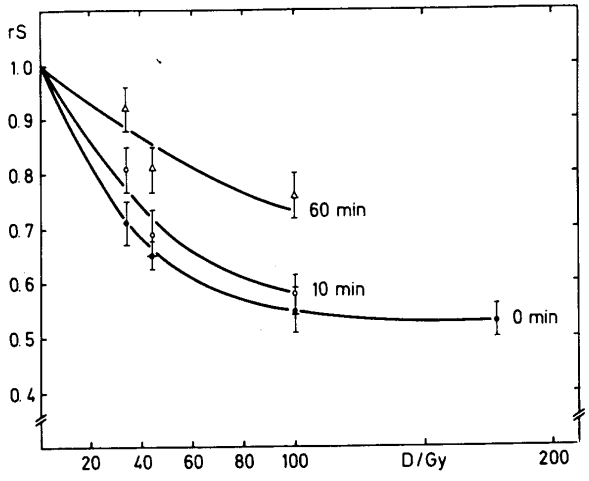

Fig. 7. Dependence of the relative sedimentation velocity (rS) of DNA subunits (V79-4 cells) upon irradiation with carbon ions for different postirradiation incubation periods; 3 experiments (from /7/).

than in subunits exposed to gamma-rays when incubated under the similar conditions (see Figure 7). We interpret this observation by the assumption that cells which contain clusters of breaks repair subunits to a lesser degree.

In order to investigate also the influence of double-strand breaks on the sedimentation behaviour of the aggregated subunits, we extended our studies to higher doses. The results are shown in Figure 8. The increase of rS in the dose range between 50 and 550 Gy exhibits a change in the subunit structure due to the induction of DNA double-strand breaks. The decrease of rS beyond doses of 550 Gy can be explained by fragmentation and degradation of DNA.

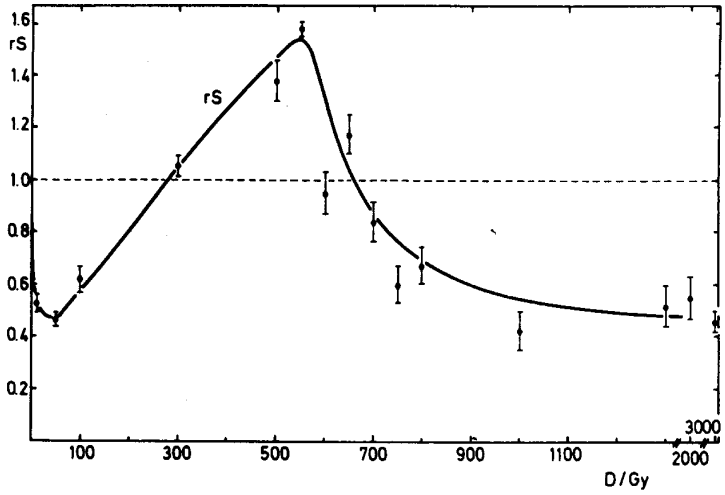

Fig. 8. Dependence of the relative sedimentation velocity (rS) of DNA subunits (V79-4 cells) on the gamma-ray dose. Number of experiments: 10 Gy, 6; 50 Gy, 5; 100 Gy, 19; 300 Gy, 9; 550-3000 Gy, 4 /7/.

This idea was supported by results obtained using different types of radiation as shown in the Figure 9. Neutrons induce more double-strand breaks than gamma-rays, resulting in an increase of rS at smaller doses. After irradiation with carbon ions the subunits are not damaged homogeneously and, therefore, the influence of double-strand breaks on rS is much less pronounced than in the case of gamma rays.

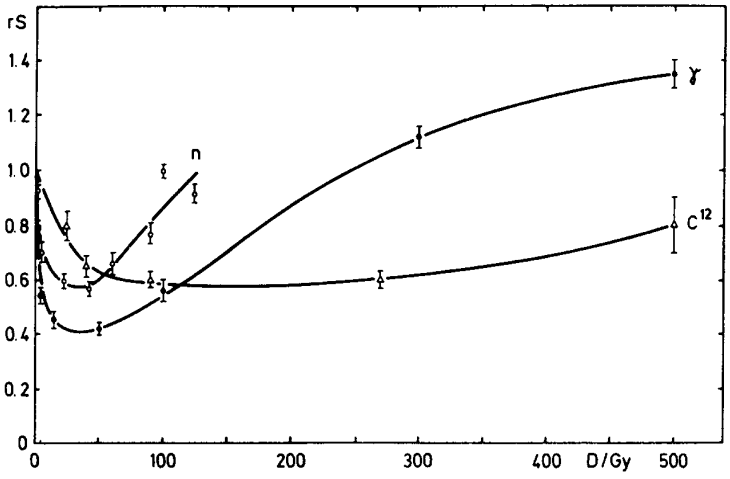

Fig. 9. Dependence of the relative sedimentation velocity (rS) of DNA subunits (V79-4 cells) on the dose of gamma-rays, neutrons and carbon ions; Number of experiments: neutrons: 4, carbon ions: 3, gamma-ray: see Figure 8 (from /7/).

If the increase of rS in the range of higher doses is caused by double-strand breaks, then it can be expected that with increasing incubation times after irradiation rS should behave as if the dose is reduced. In Figure 10 the obtained rS values are shown as a function of the incubation period after irradiation with different gamma-ray doses. At a dose of 30 Gy, at which on average less than one double-strand break per subunit is induced, the restoration of DNA subunits is based on the rejoining of single-strand breaks. In contrast, after irradiation with doses of 100 or 300 Gy rS first decreases probably due to the repair of DSBs.

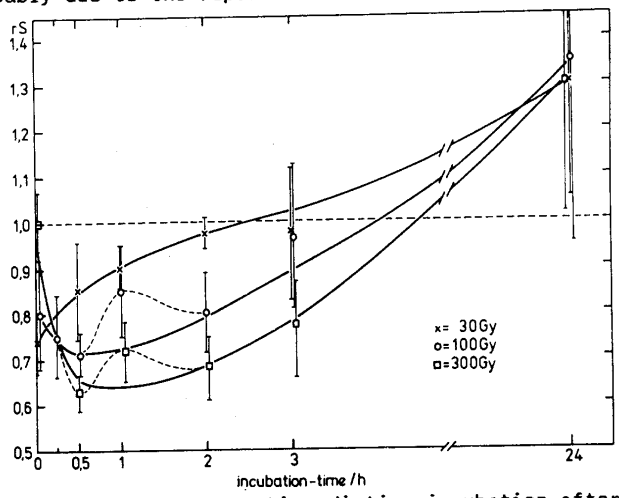

Fig. 10. The effect of a postirradiation incubation after high gamma-dose irradiation of V 79-4-cells on the relative sedimentation velocity, rS. The dashed curves should indicate that the underlying processes are propably more complex. Further experiments will be performed.

The most interesting question, however, is whether the repair of double-strand breaks leads to the undamaged structure of the subunits. We have tested this in the following way: After a high dose irradiation the cells were incubated over 24 hours. After incubation the cells were irradiated again with lower doses which influences the sedimentation only via single-strand breaks. The decrease in rS for the "repaired" subunits was compared with those of the non pre-damaged structures. Figure 11 shows that the degree of repair-mediated restoration decreases with increasing doses.

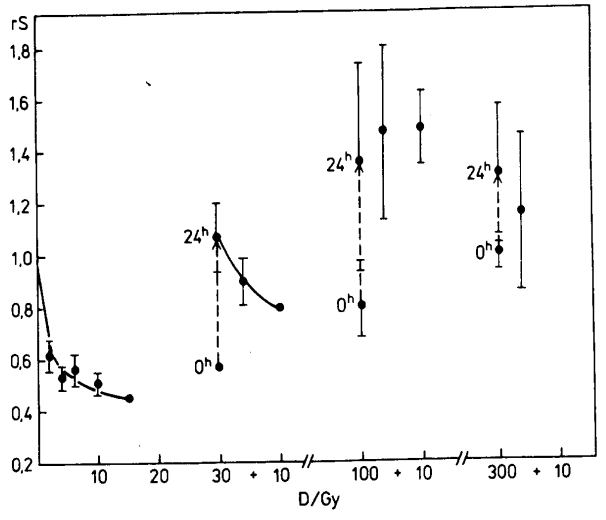

Fig. 11. The effect of a second irradiation on the relative sedimentation velocity rS of V79-4 subunits following the first high dose irradiation and an incubation period of 24 h. The experiments were performed with gamma-rays.

The data presented so far are mainly of a phenomenological character and can be summarized as follows:
The nucleoid sedimentation technique allows the investigation of repair of DNA damage and of restoration of the structure of DNA-subunits. The restoration of DNA subunit structures seems to be correct in the dose range where predominantly single-strand breaks are induced. If the number of induced double-strand breaks per DNA subunit is higher than one, the restoration of structure is incomplete.

QUANTITATIVE INTERPRETATION OF THE STRUCTURE RESTORATION

In a first approach the probability, p_o, for subunits without any single-strand breaks after irradiation is related to the radiation dose by the following equation:

$$p_o(D) = \exp(-M_o s_s D) \tag{1}$$

where D is the dose, s_s is the yield of single-strand breaks per units of molecular weight and dose, and M_o is the molecular weight of the DNA in one subunit.

From the measured rS(D), p_o can be estimated in the following manner:

$$p_o(D) = \frac{rS(D) - rS_{min}}{1 - rS_{min}} \tag{2}$$

rS_{min} represents the relative sedimentation velocity if the DNA in all subunits are relaxed due to single-strand breaks.

Differentiation of equation (2) results in

$$\frac{drS}{dD} = -M_o s_s (1 - rS_{min}) \exp(-M_o s_s D) \tag{3}$$

If predamaged subunits exist at the beginning of the second irradiation, the corresponding equation reads

$$\frac{drS_\varphi}{dD} = -M_o s_s (rS_{max} - rS_{min})(1-\varphi) \exp(-M_o s_s D) \tag{4}$$

wherein φ denotes the fraction of predamaged subunits and rS_{max} is the relative sedimentation velocity after the postincubation period following the first irradiation.
Inserting equation (4) in (3) and resolving allows the determination of

$$\varphi = 1 - \left(\frac{drS_\varphi}{dD}\right)\left(\frac{drS}{dD}\right)^{-1} \frac{1 - rS_{min}}{rS_{max} - rS_{min}} \tag{5}$$

Using experimental results which are shown in Figure 11 we have calculated the φ - values shown in Figure 12 as symbols.

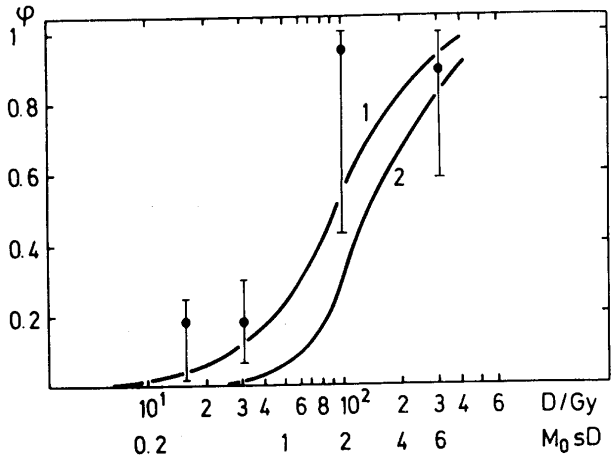

Fig. 12. Fraction φ of irrepairably damaged DNA-subunits as calculated from experimental data according to equation (5). Lines represent the results from the model described briefly in the next section, 1: for G1-, G2-, and early S-phase (equation (6)), 2: for late S-phase (equation (7)).

MODELLING OF DOUBLE-STRAND BREAK REPAIR

We have developed a model to interpret the double-strand break-repair mechanism based on the existence of DNA-subunits and on the idea that double-strand breaks within a certain subunit will or will not be repaired depending exclusively on the patterns of double-strand breaks /9/. The proposed model operates on the following assumptions:

(I) During the G1- and at the end of the G2-cell cycle stage all the subunits are attached as single subunits to the nuclear matrix.
However, during S-stage an increasing fraction of the subunits are replicated and the increasing fraction of the sister subunits are attached in pairs until the beginning of G2-stage. Therefore, in very late S-stage, the entire chromosomal DNA consists of a complete set of coupled sister subunit pairs.

(II) The question whether all of the double-strand breaks contained in a single subunit will be repaired or not is decided by the number, k, of double-strand breaks per subunit: A cell is able to repair each of their single subunits containing only one double-strand break (k=1), but it cannot repair subunits with $k \geq 2$ double-strand breaks. Using the genetic information from the homologous subunit, the repair of one double-strand break per subunit (k=1) is allowed by a recombination process, as predicted by a repair model in /10/.

(III) A cell is able to repair all the double-strand breaks in a coupled sister subunit pair if at least one of the two sister subunits has either none or only one double-strand break.

From this repair model we derive the fraction of irrepairably damaged subunits by the following equation:

$$\varphi_{mod,1} = \sum_{k=2}^{\bullet} U_k(D) \qquad (6)$$

for G1, G2 and early S-phase and

$$\varphi_{mod,2} = \sum_{k=4}^{\bullet} \left(1 - \frac{2k+2}{2^k}\right) U_k(D) \qquad (7)$$

for late S-phase respectively.

The factor $1-\frac{2k+2}{2^k}$ takes into consideration the different

distributions of k breaks in the sister subunit pairs and describes by this the probability of non-repaired double-strand breaks according to assumption (III).

The double-strand break-number distribution results from

$$U_k(D) = \int_0^\infty p_k(z) f_z(D) dz \tag{8}$$

with

$$p_k(z) = \exp(-M_o \cdot s_D \cdot z) \frac{(M_o \cdot s \cdot z)^k}{k!} \tag{9}$$

and

$$f_z(D) = \sum_{i=0}^{\infty} \exp\left(-\frac{D}{\bar{z}_f}\right) \frac{(D/\bar{z}_f)^i}{i!} f_1(z)^{*i} \tag{10}$$

$f_1(z)^{*i}$ represents the i-fold folding of the single event distribution of the specific energy z and depends on the target shape and radiation quality as well /11,12/.

The φ_{mod}-curves together with the experimental data in Figure 12, show that the model is consistent with the experimental results. It is apparent that the irrepairability of subunits increases dramatically with the dose range, where on average every subunit suffers more than one double-strand break.

CONCLUSIONS

Summarizing we can say that the experimental results are consistent with the assumed existence of DNA subunits. Furthermore the results support the hypothesis that the DNA-repair process starts in the damaged subunits independently. This means that in respect to repair processes, DNA-subunits are autonomous. The assumption that the strand break patterns in the DNA in a subunit are decisive for repair or non-repair is also consistent with the experimental results. However, it is neccessary to extend the experiments, especially with regard to variation of LET and cell cycle stage.

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