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Investigation of the SOS response of *Escherichia coli* after γ-irradiation by means of the SOS chromotest

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Summary

The kinetics of SOS system induction in *Escherichia coli* PQ37 cells by γ-irradiation has been studied by the SOS chromotest technique. It was shown that the synthesis of constitutive alkaline phosphatase is not immediately stopped in cells that suffered lethal damages from γ-irradiation. The production of DNA damages inducing the SOS system was 0.021/Gy per genome. The SOS system was switched off approximately 200 min after γ-irradiation. A correction is proposed to the calculation of the SOS system induction factor.

Agents damaging DNA in *E. coli* are known to induce the SOS reaction during which a large number of biochemical events are realized. There is a single system that controls the expression of genes involved in the SOS response. The SOS system is connected with *recA* and *lexA* genes. The most important cell properties are under the control of this system: DNA repair, mutagenesis, cell division, prophage induction of lysogens, etc. (Little and Mount, 1982; Walker, 1984).

Investigation of the SOS system can give us important information on the mechanism of action of different DNA-damaging agents on bacterial cells. For this purpose bacterial strain *E. coli* PQ37 is a very convenient object. The strain was constructed by fusion of the *sulA* and *lacZ* operons (*sulA*::*lacZ*). The expression of the *sulA* gene controlled by *lexA* and *recA* proteins can be detected by monitoring the level of β-galactosidase. Using this bacterial strain Quillardet et al. (1982) proposed the SOS chromotest technique for quantitative measurement of the action of DNA-damaging agents. The SOS chromotest is based on the simultaneous colorimetric assay of β-galactosidase and alkaline phosphatase activities.

We have already used the SOS chromotest for the investigation of SOS induction by heavy ions (Kozubek et al., 1989). One of the most important factors influencing the results was the kinetics of β-galactosidase and alkaline phosphatase synthesis. The knowledge of this kinetics is essential for the correct estimation of the genotoxic influence of different agents. We devoted the present paper to the kinetics of SOS system induction by γ-irradiation in *E. coli* PQ37 using the SOS chromotest.

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Materials and methods

Bacterial strain

We have used *Escherichia coli* K-12, the PQ37 strain with a genotype described by Quillardet and Hofnung (1985).

γ-Irradiation

The stationary-phase culture (18 h) was diluted 1 : 10 in fresh LA medium and shaken for 2 h at 37 °C to a cell density of about 10^8 cells/ml (Quillardet et al., 1982). Then the cells were diluted 10-fold in fresh LA medium and distributed into glass test tubes. After γ-irradiation with different doses the cells were incubated for an additional time at 37 °C with shaking. After different time intervals samples were taken for the assay of β-galactosidase and alkaline phosphatase.

β-Galactosidase assay

The assay has been described by Quillardet and Hofnung (1985). Cell membranes were disrupted with 0.1% SDS (sodium disulfate) in Z buffer during 10 min at room temperature. The enzyme reaction was started by adding *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (4 μg/ml in the phosphate buffer, pH = 7.0) and stopped by adding 1 M Na_2CO_3. The color reaction was measured spectrophotometrically at 420 nm with a Specol-11 spectrometer (Zeiss, G.D.R.). A comparison was made with a reference sample of cells which were not irradiated or incubated. The sample was kept on ice.

Alkaline phosphatase assay

The assay was performed as described by Quillardet and Hofnung (1985). T buffer was supplemented with 0.1% SDS to disrupt cell membranes.

Protein determination

The contents of protein in the cell suspension was measured by the Lowry method (Lowry et al., 1951).

Determination of cell survival

Cell survival was determined by the standard method, i.e., by counting the number of macrocolonies on the nutrient agar. The radiosensitivity was calculated as the slope of exponential dose–response dependences of stationary-phase cultures or as the final slope of shouldered cell survival curves of an exponential-phase culture incubated before irradiation for 2 h in fresh LA medium. Irradiation was performed in M9 buffer.

Calculation of the induction factor

The induction factor is the ratio of the normed β-galactosidase activity to the normed alkaline phosphatase activity:

$$IF(D) = \frac{g(D)/g(0)}{p(D)/p(0)} \quad (1)$$

where g(D) and g(0) are the β-galactosidase activity in γ-irradiated cells and intact cells, respectively; p(D) and p(0) are the same quantities for alkaline phosphatase. The derivative of IF(D) for the zero dose is called SOSIP (SOS induction potency).

The determination of parameters entering into different equations was performed by the minimization of the sum of deviations squared of theoretical and experimental values.

Results

Cell survival and growth curves of the cell culture after γ-irradiation

Cell survival was determined for both stationary- and exponential-phase culture (Fig. 1). The survival curve of the stationary-phase culture was exponential up to 630 Gy. The sensitivity (Do^{-1}) determined from the slope of the survival

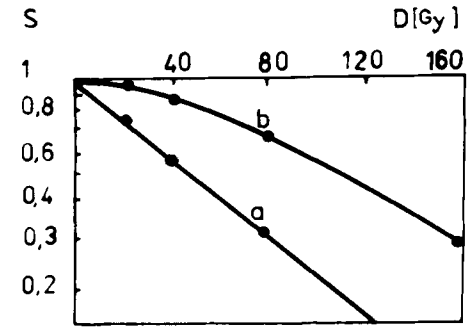

Fig. 1. The survival curve of γ-irradiated *E. coli* PQ37 cells: stationary-phase cells (a), exponentially growing cells (b); S is the cell survival (relative number), D is the γ-irradiation dose in Gy.

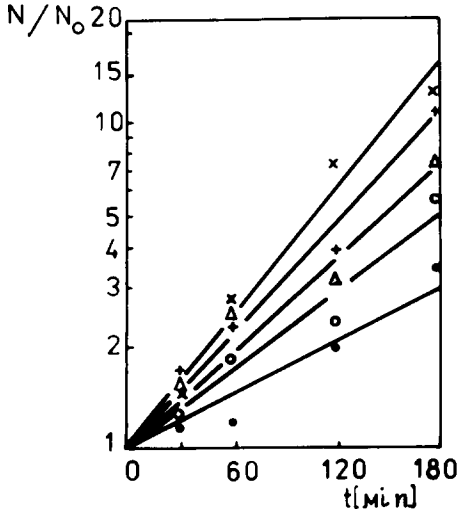

Fig. 2. The relative number of *E. coli* PQ37 cells incubated at 37 °C with shaking t min after γ-irradiation with doses 0 Gy (×), 20 Gy (+), 40 Gy (△), 80 Gy (○) or 160 Gy (●).

curve was $Do^{-1} = 0.014/Gy$, i.e., $Do = 72$ Gy. The shouldered survival curves of the exponentially growing cells could be characterized by the same sensitivity (the same final slope). The extrapolation number (n) was equal to 3. The cell survival after γ-irradiation with dose D could be represented by a function

$$S(D) = 1 - (1 - e^{-D/Do})^n \qquad (2)$$

where $Do = 72$ Gy and $n = 3$.

The number of viable cells was measured in different periods (t) of cultivation. The number of cells at a dose D and moment t was normalized to the number of cells at $D = 0$ Gy, $t = 0$ min. The averaged data of 5 experiments are plotted in Fig. 2. The data show that no delay in cell growth was observed for the doses used in our experiments (see Fig. 2). Such a phenomenon can be connected with a better efficiency of the repair processes in irradiated cells incubated under aerated conditions. This could compensate for a delay in the growth of cells at small doses.

The dependence of the slope of the growth curve $\beta_D = 1/T_o(D)$ on the γ-irradiation dose can be described by the function

$$\beta_D = \beta_o \exp(-\pi \cdot D) \qquad (3)$$

where $\beta_o = 0.0152/min$, $\pi = 0.0037/Gy$. Using equations 2 and 3 we obtain the number of cells in culture N(D,t) for different doses and different incubation times:

$$N(D,t) = S(D) \cdot \exp(\beta_D \cdot t) \qquad (4)$$

The curves generated by this equation are in good agreement with our experimental data.

Alkaline phosphatase activity in γ-irradiated E. coli PQ37

Fig. 3 represents the data on alkaline phosphatase activity in the intact *E. coli* PQ37 suspension incubated for various times. The experimental data are plotted as points while the theoretical curve corresponds to the equation

$$p(0,t) = \phi \cdot (\exp(\beta_o \cdot t) - 1) \qquad (5)$$

where $\phi = 0.068$. The formula was derived on the assumption that the rate of alkaline phosphatase synthesis is constant and so the increment of the enzyme activity dp is proportional to the number of cells in the suspension at the time t, i.e., $dp \approx \exp(\beta_o \cdot t) \cdot dt$. The coefficient ϕ depends on several biochemical parameters: the rate of alkaline phosphatase synthesis, the generation time, and the initial level of alkaline phosphatase.

The same regularities were also observed in the case of general protein contents in the cell cultures (the right scale in Fig. 3). So phosphatase synthesis occurs in parallel to protein synthesis in the exponentially growing intact cells. It should be mentioned that there is no strict correlation be-

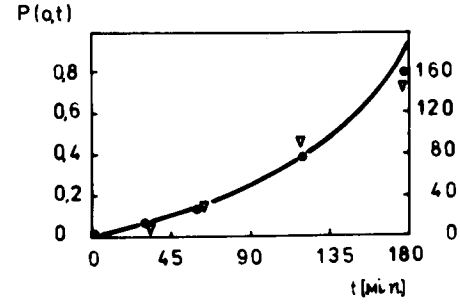

Fig. 3. The activity of alkaline phosphatase P(0,t) (●, left scale) and of the protein amount (▽, right scale) in a suspension of intact *E. coli* PQ37 cells aerated for t min at 37 °C. The theoretical curve was calculated according to Eq. 5.

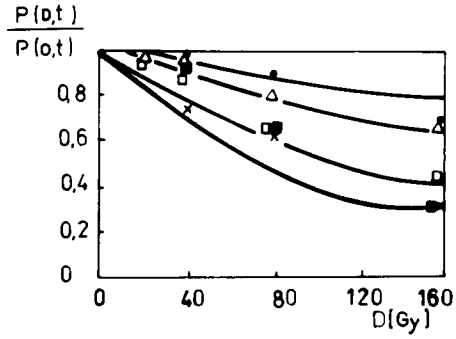

Fig. 4. The relative activity of alkaline phosphatase in a suspension of *E. coli* PQ37 cells P(D,t)/P(0,t) as a function of the γ-irradiation dose D after aeration for t = 30 min (●), 60 min (△), 120 min (□); the same scale for protein t = 120 min (■), 180 min (×). The theoretical curves were calculated according to Eq. 6.

tween the measured activity of alkaline phosphatase and the density of cells in the suspension sample. Such a correlation, however, arises for greater time periods as can be seen from Eq. 5.

Alkaline phosphatase activity was measured in different intervals of incubation of the γ-irradiated cells. The experimentally obtained ratios p(D, t)/p(0,t) are given as the points in Fig. 4. It can be seen that these ratios decrease as the γ-irradiation dose increases. Subsequent incubation of irradiated cells enhances this tendency.

The equation

$$\frac{p(D,t)}{p(0,t)} = \frac{(S/\beta_o)\cdot(\exp(\beta_D\cdot t)-1)+(1-S)\cdot t}{(1/\beta_o)\cdot(\exp(\beta_o\cdot t)-1)} \qquad (6)$$

satisfactorily describes the obtained dependence of alkaline phosphatase activity on γ-irradiation dose and incubation time. The first term describes the phosphatase activity of dividing cells and the second gives the enzyme activity of γ-damaged cells that are unable to form colonies. Good agreement of Eq. 6 with the experimental data allows us to formulate the following hypothesis. In lethally damaged cells alkaline phosphatase synthesis is not stopped; its rate remains constant during the whole experiment (180 min).

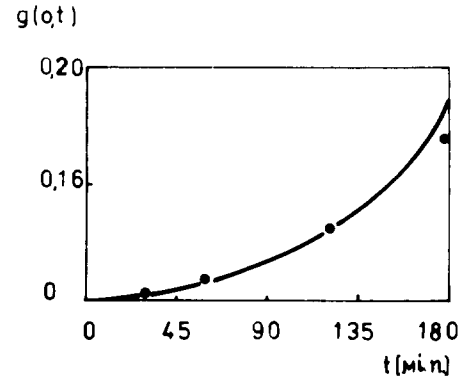

Fig. 5. The β-galactosidase activity g(0,t) of intact *E. coli* PQ37 cells during aeration for t min. The theoretical curve was calculated according to Eq. 5.

The dependence of β-galactosidase activity on γ-irradiation dose and incubation time

Fig. 5 represents the experimental data on β-galactosidase activity in the suspension of intact cells incubated with shaking at 37 °C. The inducible enzyme activity of exponentially growing cells (180 min) increases in a way similar to that of the constitutive enzyme activity of alkaline phosphatase. Therefore, the time dependence can be described by the same equation (Eq. 5). The average rate of enzyme synthesis could be connected with some background level of the enzyme or with a constant probability of SOS induction (per cell per time unit).

The experimentally obtained ratios g(D,t)/g(0,t), where g(D,t) is the activity of the

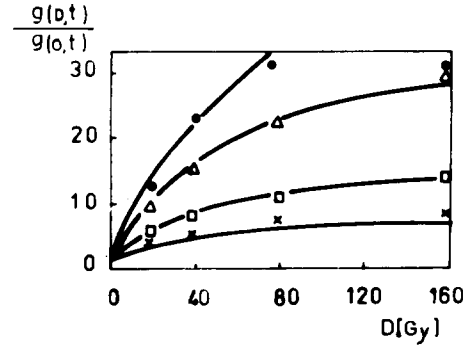

Fig. 6. The relative activity of β-galactosidase in a suspension of *E. coli* PQ37 cells g(D,t)/g(0,t) as a function of the γ-irradiation dose D after aeration for t = 30 min (●), 60 min (△), 120 min (□) or 180 min (×). The theoretical curves were calculated according to Eq. 5 and Eq. 7.

enzyme at dose D and time t, are given as the points in Fig. 6. The data show that the enzyme activity is sharply increased with an increase of the γ-irradiation dose. Then the dependence is saturated at approximately 100 Gy. The normed β-galactosidase activity decreases during the incubation.

The obtained dependence allows 2 interpretations. In the first the efficiency of SOS system induction depends on the amount of damage in each individual cell. According to the second interpretation the enzyme activity increases proportionally to the number of damaged cells (the enzyme activity in the induced cells remains constant).

It seems impossible at the present time to determine the enzyme activities in individual cells. We shall use the second interpretation which allows a mathematical description and show that it leads to a good description of the experimental data.

Let $\alpha \cdot D$ be the yield of DNA damages inducing the SOS system. Then $\exp(-\alpha \cdot D)$ will give the number of undamaged cells, and $1 - \exp(-\alpha \cdot D)$ will represent the number of damaged cells. The β-galactosidase activity will be proportional to the last term and will have a plateau at a higher dose.

The repair process that occurs during incubation has a stochastic nature. If δ is the probability that the cell will be repaired per time unit, we can expect that $\exp(-\delta \cdot t)$ will give the fraction of cells that are able to accomplish the inducible repair during time interval (0,t). Therefore the dependence of the β-galactosidase activity on the γ-radiation dose and incubation time will have a form

$$g(D, t) = a \cdot (1 - \exp(-\alpha \cdot D)) \cdot (1 - \exp(-\delta \cdot t)) \quad (7)$$

where a is the proportionality constant. The theoretical curve agrees with experimental data points for the following values of the parameters: $a = 0.1783$; $\alpha = 0.021/\text{Gy}$; $\delta = 0.005/\text{min}$.

These data permit us to determine the dose which causes on average 1 SOS-inducing injury per cell: $D^{SOS} = 48$ Gy. The SOS system is switched off approximately 200 min after γ-irradiation.

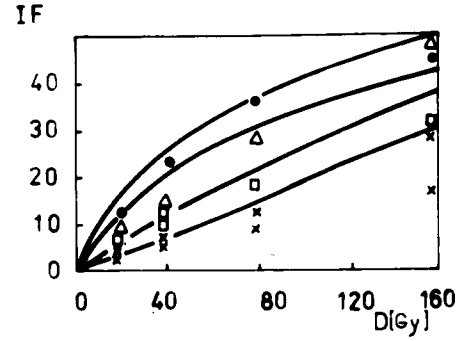

Fig. 7. The value of the induction factor (IF) calculated according to Eq. 1 as a function of the γ-irradiation dose for *E. coli* PQ37 cells incubated for different periods of time: 30 min (●), 60 min (△), 120 min (□), 180 min (×) (the data are normalized to phosphatase and protein). The theoretical curves were calculated according to Eqs. 5–7.

Induction potency of the SOS system

The induction factor (IF) has been calculated (Quillardet et al., 1982) as the ratio of the β-galactosidase and alkaline phosphatase activities in a suspension of cells incubated for 120 min (Eq. 1). We have obtained values of IF(D,t) for different periods of incubation after γ-irradiation. Fig. 7 shows the experimental points and the theoretical curves generated by equations 6 and 7. The theoretical and experimental IF values agree at medium and high doses for different time intervals; agreement is not so good at low doses. It

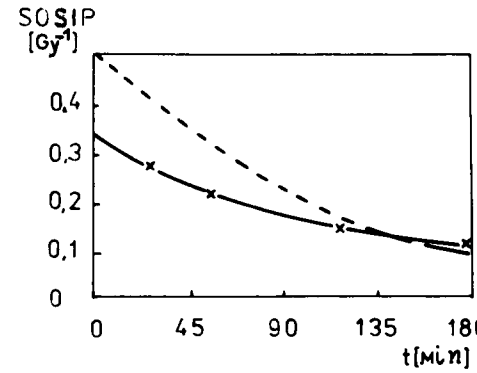

Fig. 8. The SOSIP values plotted versus the incubation time (solid line), and the theoretical curve generated by Eqs. 6 and 7 (dashed line).

should be noted that the fluctuation of IF values is less than that of the enzyme activity values, because of their statistical correlation.

The induction potency of the SOS system is characterized by the initial slope of the IF(D) curve. Fig. 8 shows the time dependence SOSIP(t) obtained experimentally (solid line) and calculated from equations 6 and 7 (dashed line). As can be seen, the values of SOSIP decrease with cell incubation time. Comparison of the experimental and theoretical curves reveals better agreement at a longer incubation time.

Discussion

The data obtained allow the following conclusions.

The sensitivity of the PQ37 strain of *E. coli* to γ-irradiation in both stationary and exponential growth phases is equal to $Do^{-1} = 0.014/Gy$. If the cells had been additionally incubated before irradiation, the exponential survival curve acquired a shoulder with the extrapolation number equal to 3.

It follows from the experimental data that constitutive alkaline phosphatase and total protein are synthesized at approximately equal rates in both intact and irradiated cells.

The dependence of the activity of irradiated cells on the incubation time is well described by Eq. 6 only if all cells, including the lethally damaged ones, are taken into account. This important observation indicates that the synthesis of constitutive alkaline phosphatase continues for some time after the cell is lethally damaged by irradiation.

The activity of inducible β-galactosidase sharply increases during the incubation after γ-irradiation. The detected increase in activity of this enzyme during incubation of intact cells can be connected with some background level of the enzyme in each cell or with spontaneous SOS induction.

The increase in activity of β-galactosidase after γ-irradiation is shown to depend strictly on the dose and consequently on the amount of damage in cells or on the number of damaged cells. This dependence indicates that the damage induced by γ-radiation is a substrate for triggering of the SOS system in *E. coli* cells.

It follows from the experimental data and calculations that the yield of damages triggering the SOS system is 0.021/Gy per cell, and the time of the SOS system switching off is on average 200 min.

The induction factor is the quotient of the relative activities of the inducible system (β-galactosidase) g(D,t)/g(0,t) and the constitutive enzyme (alkaline phosphatase) p(D,t)/p(0,t). The constitutive enzyme activity p(0,t) and p(D,t) is mainly associated with the number of cells including those lethally damaged. The β-galactosidase activity in intact cells also grows exponentially (Fig. 5) in accordance with the exponential increase in the number of cells (Fig. 1).

To calculate IF, g(D,t) is the most essential quantity. After γ-irradiation with a sufficient dose most cells suffer damage, which inhibits their division. Moreover, the number of non-lethally damaged cells remains at the same level, until the SOS system is switched off (200 min). Therefore, β-galactosidase activity depends on the number of damaged cells at $t = 0$ rather than on the number of cells at $t = 120$ min, i.e., it is related to the number of cells at the initial moment of time.

It is reasonable to measure the β-galactosidase activity of a suspension with intact cells before the cells have divided, i.e., at a moment μ shorter than the doubling time. The most suitable value of μ is the one that reflects, on the one hand, the number of cells during irradiation and, on the other hand, the SOS system induction in them. This time should be less than the cell generation time T_o. For example, if $T_o = 46$ min (as in our case), μ can be taken as equal to 30 min.

The correction factor t/μ must be introduced into the final calculation of IF:

$$IF = \frac{\beta(t,D)/\beta(\mu,0)}{p(\mu,D)/p(\mu,0)} \cdot \frac{t}{\mu} \qquad (8)$$

where t and μ are the incubation periods. We are of the opinion that this yields a more accurate IF for the comparative analysis of action of different mutagens. The proposed correction is especially significant for dealing with mutagens whose presence inhibits cell growth. In this case the value of IF will be a more reliable indication of the damage to DNA.

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