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# Detection of ionizing radiations with the SOS Chromotest, a bacterial short-term test for genotoxic agents

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## Summary

The effects of 3 types of ionizing radiation, γ-rays, neutrons and accelerated α-particles, were examined using the SOS Chromotest, a bacterial colorimetric assay for genotoxic agents based on the measurement of the SOS response in *Escherichia coli*.

The SOS Chromotest appeared to be a sensitive and simple assay to detect quantitatively these radiations as well as their biological effects. The range of adsorbed doses for which induction was observed was similar for the 3 types of radiation, the minimum inducing doses being in the order of 2.5–5 Gy. We discuss the possible use of these observations to study the molecular action of radiations and to compare their genotoxic effects with those of chemicals.

A number of bacterial short-term tests have been developed in the past 15 years to detect genotoxic chemicals. Many of these tests allow a quantitative evaluation of the genotoxic activity of the compounds tested (Hollstein and McCann, 1979; Hofnung and Quillardet, 1986). On the other hand, the DNA-damaging activities of radiations and their consequences, such as mutagenesis, carcinogenesis and cell death, have been studied in great detail (Upton, 1974; Regan and Setlow, 1974; reviews in Paterson and Gentner, 1984; Gentner and Paterson, 1984). Although several studies on the effects of radiations in bacteria have been performed in the past (Munson and Bridges, 1964, 1973), there have been relatively few studies with radiations using standardized and validated bacterial short-term tests as an experimental system (Ames, 1972; Heddle and Bruce, 1977; Imray and MacPhee, 1981; Levin et al., 1982; Roos et al., 1985).

It thus appeared interesting to us to evaluate the capacity of a bacterial short-term test, the SOS Chromotest (Quillardet et al., 1982), to detect radiations. The principle of this test consists in monitoring expression of gene *sfiA* belonging to the SOS system, a group of functions induced by

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DNA damage (Quillardet et al., 1982). The SOS Chromotest has already been validated with a large number of chemical agents (Ohta et al., 1984; Quillardet et al., 1985). In a previous study, we showed that the SOS Chromotest efficiently detected the SOS-inducing effect of UV-light, a non-ionizing radiation (Quillardet and Hofnung, 1984). We found that, if, as is generally believed, pyrimidine dimers (a major UV lesion) are responsible for SOS induction, a very small number of them (if not one) per cell was enough to trigger the response. The SOS Chromotest therefore appeared very sensitive to detect this type of radiation.

In the present work, using the SOS Chromotest, we measured the SOS-inducing capacity of various types of ionizing radiation, i.e., $^{60}$Co γ-rays, α- and fast neutron particles.

## Materials and methods

*Bacterial strain, media and buffers*

The experiments described here were performed using the *E. coli* K12 strain PQ37, the standard tester strain for the SOS Chromotest (Quillardet et al., 1982; Quillardet and Hofnung, 1985).

L medium and 63 buffer have been previously described (Miller, 1972). Buffers and reagents for alkaline phosphatase and β-galactosidase assays have been described elsewhere (Quillardet et al., 1982; Quillardet and Hofnung, 1985).

*Irradiation procedure and radiation sources*

Exponentially growing bacteria, cultivated in L medium supplemented with 20 μg ampicillin/ml, at a concentration of $2-5 \times 10^8$ cells/ml, were centrifuged, washed, and suspended in 63 buffer. The bacterial suspension was vigorously aerated just before irradiation. Samples of 1 ml of cell suspension were irradiated, at room temperature, in polystyrene photometric cuvettes disposed transversally to the radiation beam through a plastic device. The plastic cuvette walls were 1 mm thick, and the sample thickness was 4 mm. For γ and neutron irradiation a plastic shield was positioned in front of the sample in order to ensure the electronical equilibrium in the irradiated sample. For dose measurements, the biological sample was withdrawn and a dosimeter was positioned at the same place.

*Gamma-rays (photons)*

The samples were irradiated by the use of a $^{60}$Co cobalt therapy unit which allows a good irradiation geometry. The irradiation room is large enough to minimize scattered radiations. The dose rate was 0.5 Gy/min as determined by ferrous sulfate dosimetry, according to the previously described procedure (Ricourt et al., 1975; Mijnheer et al., 1984).

*Neutrons*

The neutrons were produced by 34-MeV proton on a thick beryllium target at the Orleans cyclotron (CNRS-CHRO). Details of this facility have been reported elsewhere (Pihet et al., 1987).

Dosimetry was performed according to the protocol previously described (Ricourt et al., 1975; Mijnheer et al., 1984) with the help of tissue-equivalent (TE) and aluminum ionization chambers. The dose rate achieved with this set-up was 5 Gy/min for an incident proton beam current of 12.7 μA. The indicated doses do not include the γ-ray contribution which was about 1.1% of the neutron doses.

*Accelerated helions*

The beam of accelerated helions, 650 MeV/μm, was produced at the synchro-cyclotron Saturne II (Saclay Nuclear Research Center). The experimental set-up and dosimetry technique have been described previously (Chemtob et al., 1975; Nguyen et al., 1981). The irradiations were performed in the plateau portion of the Bragg curve. The average dose rate was about 2.5 Gy/min.

*SOS Chromotest*

The SOS Chromotest was performed as described (Quillardet et al., 1982; Quillardet and Hofnung, 1985) except that induction was done by irradiation instead of exposure to a chemical. We used the same parameters to follow the expression of gene *sfiA* as with chemicals, except that doses were expressed in radiation units of absorbed dose (Gy) instead of product concentrations.

In brief, after irradiation, bacterial suspensions were diluted in L medium, incubated at 37°C for

an appropriate period of time, and then assayed for alkaline phosphatase and β-galactosidase activities as previously described. To monitor accurately *sfiA* rate of expression after irradiation, we compared β-galactosidase activity (B), which represents *sfiA* expression, with alkaline phosphatase activity (P), which is assumed to account for general protein synthesis during the incubation period. The ratio R = B/P is, under these conditions, a measure of β-galactosidase-specific activity. This activity ratio (R) at dose x, R(x) = B(x)/P(x), was normalized to its value in unirradiated cells, R(0) = B(0)/P(0). It was called the induction factor, I(x) = R(x)/R(0) (Quillardet et al., 1982). Under our assumptions, the induction factor, I, is proportional to the specific activity of β-galactosidase in the tester strain and reflects the rate of *sfiA* gene expression. The SOS inducing potency (SOSIP) is the slope of the linear region of the dose–response curve. It represents the induction factor per unit absorbed dose of the radiation assayed.

*Bacterial survival*

After irradiation, samples of the irradiated bacterial suspension were diluted in 63 buffer and plated on L agar plates. Colonies were scored after 24 h of incubation of the plates.

## Results

The SOS Chromotest (Quillardet et al., 1982; Quillardet and Hofnung, 1985) allows us to measure the ability of a compound to induce the expression of gene *sfiA* in *E. coli*. Gene *sfiA* is under the control of the LexA protein. This protein is the general repressor of the SOS system, a set of genes induced in response to a wide variety of DNA damages (see for review Walker, 1984). The expression of gene *sfiA* is easily monitored thanks to a genetic fusion between *sfiA* and *lacZ*, the structural gene for β-galactosidase in *E. coli*. In this assay the genotoxic activity of each chemical compound is characterized by a single parameter, its SOS inducing potency (SOSIP) (Materials and methods; Quillardet and Hofnung, 1985), which reflects its ability to induce the expression of gene *sfiA*. The SOSIP measures the increase in the rate of expression of the *sfiA* gene (induction factor) per unit dose of compound in the case of chemicals, or per unit of absorbed dose in the case of radiation (Materials and methods).

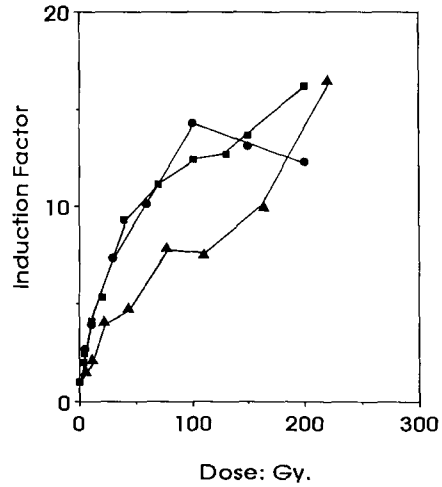


Fig. 1. Dose–response curves for accelerated α-particles (■), accelerated neutrons (●) and γ-rays (▲). The induction factor is the ratio of β-galactosidase activity to alkaline phosphatase activity at a given dose of irradiation normalized to its value in unirradiated cells (see Materials and methods).

The 3 types of ionizing radiation examined in the present work, γ-rays, fast neutrons and accelerated α-particles, all gave a positive response in the SOS Chromotest (Fig. 1). The range of absorbed doses for which induction was observed

TABLE 1

BACTERIAL SURVIVAL AND SOS INDUCTION AFTER α-, NEUTRON- AND γ-IRRADIATION

| | 50% survival (Gy) | SOSIP (per Gy) | MID (Gy) |
|---|---|---|---|
| Gamma | 45 | 0.12 | < 5 |
| Neutron | 50 | 0.22 | 2.5 |
| Alpha | 20 | 0.28 | 2.5 |

The 50% survival dose is the dose of radiation at which 50% of the bacterial population is killed. The number of viable cells was measured by plating appropriate dilutions of the bacterial suspension on Luria broth agar medium and scoring the number of colonies after 24 h of incubation at 37 °C.

The SOSIP (SOS induction potency) is the slope of the dose–response curve representing the induction factor versus the dose of radiation (see: text and Fig. 1).

The MID (minimum inducing dose) is the lowest dose at which the induction factor is systematically over twice the background.

was similar for the 3 types of radiation. The minimum inducing doses (MID) were very similar ranging from 2.5 Gy for $\alpha$ and neutron particles to less than 5 Gy for $\gamma$-rays (Table 1). The induction factor reached a maximum level of 14, at a dose of 100 Gy, for neutron particles and of more than 16, at a dose of about 200 Gy, for both gamma rays and $\alpha$-particles (Fig. 1). The SOSIP for the 3 types of radiation differed by a factor of less than 2.5 (Table 1). These differences were not considered significant. Similar differences were obtained when bacterial survival was measured: the doses killing 50% ($LD_{50}$) of the bacterial population were also in the same range, 20 Gy for accelerated $\alpha$-particles, 45 Gy for $\gamma$-rays and 50 Gy for neutron particles (Table 1).

## Discussion

The results presented show that 3 types of ionizing radiation, $\gamma$-rays, accelerated $\alpha$-particles and neutron particles, are inducers of the *sfiA-lacZ* fusion. Although it was already known that ionizing radiation could evoke some SOS responses such as induction of phage $\lambda$ or cell elongation (Munson and Bridges, 1964, 1973), the results presented here provide a very simple means to detect quantitatively these radiations as well as their biological effects. We will discuss briefly 4 aspects of these observations.

(1) The signal obtained in the SOS Chromotest is essentially related to the absorbed dose and not to the nature of the radiation examined. Indeed, the minimum inducing doses, SOS inducing potencies and lethal effects were comparable in the 3 cases. This shows that the effects produced by these radiations depend essentially on the energy absorbed by the sample and not on the exact nature of the radiations. Such a conclusion would be compatible with the idea that ionizing radiations are relatively non-selective with respect to energy deposition, which relies essentially on electron density and electron binding energy in the target (Gentner and Paterson, 1984).

(2) In addition to its simplicity, the SOS Chromotest is very sensitive to detect ionizing radiations. In the SOS Chromotest, the MID, the lowest dose at which the response is systematically over twice the background, was 2–5 Gy with the 3 types of radiation assayed. They correspond to the lowest doubling doses which were detected in the Ames test (see below). In addition, a dose-response relationship was obtained in which the induction factor reached values over 15-fold the background (Fig. 1) as is usually observed with SOS-inducing chemicals.

Ionizing radiations have been shown to induce mutations in a variety of biological systems (Ward, 1975). However, several contradictory reports with the *Salmonella typhimurium* short-term test have been published. As early as 1972, Ames reported that X-rays and fission neutrons produced a positive response (Ames, 1972). Later, Heddle and Bruce (1977) reported a negative Ames test response to $\gamma$-irradiation. However, Imray and MacPhee (1981) showed that 2 of the Ames tester strains responded to $\gamma$-irradiation although doses of 200 Gy of $\gamma$-rays were required to triple the spontaneous reversion rate. More recently, Isildar and Bakale (1984) found a positive response of various Salmonella tester strains to X-rays and $\gamma$-rays. Mutation doubling doses from more than a few hundred Gy down to 26 Gy were found for the plasmid-free strains (TA1535, 1537, 1538), and doubling doses between 28 and 7 Gy were found for the same strains containing the pKM 101 plasmid (TA100, 2637, 98). Two other tester strains, TA102 and TA2678, with an enlarged number of *his*$^-$ sites due to the insertion in bacteria of a multicopy plasmid carrying the mutated *his* gene, have been reported to have a doubling dose for reversion of about 6 Gy after X-rays (Levin et al., 1982). Modifying the standard Ames test procedure, by delaying the irradiation for 6 h after the beginning of the incubation of the plated bacteria, Roos et al. (1985) could measure a low mutation doubling dose of 1–2 Gy in response to $\gamma$-irradiation.

Thus, the doses of radiation at which mutations are detected vary largely from one study to another. These discrepancies may be due to the genetic background of the bacterial strains and the markers monitored or to the differences between test protocols (e.g., delayed irradiation, irradiation in liquid or solid media). However, they could also be due to the fact that the number of mutants induced by irradiation always remains low (i.e., less than 4 times the spontaneous mutation rate in

the above-mentioned studies), thus decreasing the test sensitivity. At any rate, as mentioned above, the lowest doubling doses found in the Ames test are comparable to the doubling dose found in the SOS Chromotest.

(3) Although the major cause of SOS induction by ionizing radiations is certainly DNA damage (Gentner and Paterson, 1984), the exact nature of the inducing lesions remains to be determined.

In addition to oxidative damage to DNA bases (Gentner and Paterson, 1984) an important biological consequence of ionizing radiation on DNA is the formation of strand breaks; these may be one of the consequences of the oxidative lesions (Ward, 1975). Single-strand breaks (SSB) as well as double-strand breaks (DSB) may arise in irradiated DNA. DSB are potentially the most dangerous of these lesions, since, if they are not correctly repaired, they may lead to extensive loss or rearrangement of genetic material with serious consequences for the cell, including death. Ionizing radiations can produce 2 kinds of DSB: (i) direct DSB in which the covalently linked backbone of the DNA is broken at the time of exposure to radiations and (ii) indirect DSB, due to a wide variety of damages which lead to DSB via the action of certain enzymes (Bryant, 1986).

In *Escherichia coli*, the repair of DSB requires an active *recA* gene, the presence of another DNA duplex that has the same base sequence as the broken double helix (Krasin and Hutchinson, 1977) and the synthesis of proteins controlled by the *lexA* gene product, the repressor of the SOS genes (Krasin and Hutchinson, 1981). The product of gene *recA* involved in repair of DSB, and the *ruv* gene product, expected to be involved in repair of DSB (Lloyd et al., 1984), belong to these *lexA*-controlled SOS activities. Thus, since several SOS activities seem to be involved in the repair of DSB and since these lesions are produced by ionizing radiation, it is not surprising that ionizing radiation efficiently triggered the SOS system. Indeed a lesion frequently induces its own system of repair (Walker, 1984).

We and others have recently described a number of strains, derived from the SOS Chromotest tester strain PQ37, which can be used for the diagnosis of DNA lesions (Boiteux et al., 1984; Salles et al., 1987; Quillardet and Hofnung, 1987, 1988). The observation that PQ37 responds well to ionizing radiations opens the way for the use of these derivatives to study the lesions responsible for induction and to test the role of strand breaks or other lesions. The approach consists in examining the effects of specific repair mutations on the induction of the *sfiA-lacZ* fusion. In particular, it will be interesting to evaluate the effects of a *ΔoxyR* mutation, which affects the response to oxidative agents, on the SOS response induced by radiations.

It is likely that the SOS response may be induced by most or all types of DNA damages due to ionizing radiations (DSB, SSB and base damages) provided that they affect replication. Since in this assay bacterial survival is not required, even unrepaired DSB, a lethal event, should participate in the response. In contrast, since DNA strand breaks lead to cell death and since their repair, which involves recombinative events, is expected to be error-proof, they do not lead to the appearance of mutants and therefore to detectable signals in mutagenesis assays (see Friedberg, 1985). Thus, for detecting DSB a major lesion produced in various conditions by ionizing radiations, measuring the induction of the SOS response may be more relevant than measuring mutation frequencies.

(4) Finally, the SOS Chromotest provides a supplementary system to compare the genetic effects of radiations and chemicals. Its high sensitivity to detect the genotoxic effects of ionizing radiations as well as of chemicals and its practical advantages (simplicity, rapid response) indicate that it could be a useful system to compare the effect of radiations and of chemicals. Because of the wide body of knowledge on the biological effects of radiations, attempts have been made to relate quantitatively these effects to those of chemicals (Bridges, 1973; Crow, 1973; Committee 17, 1975; Latarjet, 1977; Hussain and Ehrenberg, 1977). One of the ways to compare these effects consists in calculating the radiation dose which produces the same quantitative response as an exposure to a chemical (concentration × time) in a given test system. Since a large data base of quantitative values is available for the activity of chemicals in the SOS Chromotest, it becomes possible to use this test to define doses of chemicals

which yield comparable effects to certain doses of radiations (Latarjet, 1977, 1988). For example, one may calculate in this system that the signal detected after exposure for 2 h to 1 nmole of ethyl methane sulfonate corresponds to that produced by 1 Rad.

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