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Ozone Resistance of Radiosensitive Strains of *Escherichia coli* K-12

Michel Harvey, Young Sup Chung and Yong-Keel Choi*
Dept. of Biology, Univ. of Montreal, Canada
**Dept. of Biology, Hanyang University*

Escherichia coli K-12 방사선 감수성 균주의 오존 내성

Michel Harvey·정영섭·최영길*
카나다 몬트리얼대학교 생물학과
**한양대학교 자연대학 생물학과*

ABSTRACT: Ozone, an atmospheric pollutant, can damage similar UV and X-rays DNA and its components. It is possible then that the DNA damage produced by this gas are similar, to some extent, to those of radiations and that they could be repaired by the same DNA repair mechanisms. It has been observed in *Escherichia coli* that radiosensitive strains such as *lex* A, *rec* A and *pol* A, all deficient to some extent for DNA repair, are more sensitive to ozone than a wild type strain. We have then determined the ozone resistance and host-cell reactivation of ozone-damaged T3 phages for the *E. coli* double mutants *pol* A, *lex* A, *uvr* B, *lex* A, *uvr* A, *rec A* and *rec* A *lex* A. According to the results, the DNA polymerase 1 plays a key role in ozone resistance and Type 11 mechanism and/or short patch excision repair are the most important for it. The interactions between the different DNA repair mechanisms are secondary. There is a strong correlation between ozone resistance and the capacity to reactivate T3 phages damaged by ozone.
KEY WORDS: Ozone, DNA repair, DNA damage, *Escherichia coli.*

Ozone is known as a major component of air pollution (Goldstein 1980) and due to its presence in the atmosphere several studies were undertaken to understand the mechanisms of this gas at the cellular level on man and other organisms.

Over the years considerable data has been accumulated indicating that ozone is a radiomimetic agent (Lamberts *et al.* 1964; Hoigne and Bader 1975; Hamelin and Chung 1976) as well as a mutagenic agent (Zelac *et al.* 1971a,b; Hamelin and Chung 1974a; L'Herault and Chung 1984). Furthermore, ozone can damage DNA (Shinriki *et al.* 1984; Hamelin (1985); Sawadaishi *et al.* 1985) and both the base and sugar moieties (Ishizaki *et al.* 1981, 1984; Sawadaishi *et al.* 1986). This gas can also induce DNA degradation in mucoid and DNA polymerase I mutants of *Escherichia coli* K-12 (Hamelin *et al.* 1977a,b). It is then possible that ozone and ionizing radiations may produce similar damages that could be repaired, at least to some extent, by the same DNA repair mechanisms.

To verify this hypothesis, investigations were undertaken in this laboratory with *Escherichia coli* mutants deficient in some of the DNA repair mechanisms. In agreement with the hypothesis, it was shown that the radiosensitive strains *lex* A (Hamelin and Chung 1974a) *rec* A and especially *pol* A (Hamelin and Chung 1978) were more sensitive to ozone than the wild type. We have then determined the ozone resistance of the double mutants *pol* A *lex* A, *uvr* B *lex* A, *uvr* A *rec* A and *rec* A *lex* A to further specify which are the impor-

tant DNA repair mechanisms in ozone resistance and to see if interactions between them are possible. We have also determined the host-cell reactivation capacity of these mutants to T3 phages damaged by ozone, to see if a correlation exists between ozone resistance and the capacity to reactivate phages.

MATERIALS AND METHODS

Bacterial strains

The different strains of *E. coli* used in this study are listed in Table 1.

Media

The bacteria were grown in NB broth consisting of beef extract 3g and 5g peptone per liter of distilled water. For NB agar, the medium was solidified by the addition of 1.5% Bacto agar (Difco). To get soft NB agar, 0.7% Bacto agar was added.

Ozone treatment of bacterial strains

Log phase cultures (5×10^8 bacteria/m*l*) were prepared and the ozone treatment was done by direct bubbling 50 ppm of ozone for 30 minutes

Table 1. *List of E. coli strains used*

Strain	Relevant genotype	Chromosomal markers	Source
AB1157		*thi* -1, *arg* E3, *his* -4, *pro* A2, *leu* -6, *thr* -1, *lac* Y1, *gal* K2, *ara* -14, *mtl* -1, *xyl* -5, *str* -31, *tsx* -33, *sup* E44	B.J. Bachmann
AB1886	(*uvr* A)	*uvr* A6 derivative of AB1157	P. Howard-Flanders
AB2463	(*rec* A)	*rec* A13 derivative of AB1157	P. Howard-Flanders
AB2480	(*rec* A *uvr* A)	*uvr* A6, *rec* A13, *thi* -1, *pro* A2, *lac* Y1 or *lac* Z4, *gal* K2, *str* A8 or *str* A31 *tsx* -33, *sup* E44	P. Howard-Flanders
DM49	(*lex* A)	*lex* -3 derivative of AB1157	D.W. Mount
DM823	(*rec* A)	*his*$^+$, *rec* A56 derivative of AB1157	D.W. Mount
DM824	(*rec* A *lex* A)	*lex* -3, *his*$^+$, *rec* A56 derivative of AB1157	D.W. Mount
DY98		W3110 *lac* Z, *thy*$^-$, *mef* E, *str* -r	K.C. Smith
DY99	(*lex* A)	*lex* A1 derivative of DY98	K.C. Smith
DY100	(*pol* A)	*pol* A1 derivative of DY98	K.C. Smith
DY101	(*pol* A *lex* A)	*pol* A1, *lex* A1 derivative of DY98	K.C. Smith
DY146	(*lex* A *uvr* B)	*lex* A1, *uvr* B5, W3110 *lac* Z, *thy*$^-$, *rha*$^-$, *leu* B *met* E	K.C. Smith

at a constant rate of 2.1 *l*/min. according to the method described by Hamelin and Chung (1974b). At 5 min. intervals a sample is taken from the culture and proper dilutions are spread on NB agar plates. The plates are incubated 24 to 48 hours at 37 °C and the survival fraction determined.

Host cell reactivation of ozone treated T3 phages

The procedure has been described previously (L'Herault and Chung 1982). T3 phage stock suspensions were prepared by the confluent lysis method (Adams 1959) with the strain DY98. Water suspensions (10m*l*) containing 10^6 phages/m*l* were exposed to 10 ppm of ozone for 10 minutes as described above. Samples (0.1 m*l*) were taken from the phage suspensions, before and after ozone treatment, and proper dilutions were mixed with 0.1 m*l* of each bacterial strain in melted soft NB agar. The soft agar is then poured on NB agar plates. The plates were incubated for 24 hours at 37 °C. The survival fraction (S_{10}/S_0) and the reactivation factor (survival fraction with tested strain/survival fraction with control strain × 100) were determined for all the strains used.

RESULTS

Survival curves of *E. coli* strains after ozone exposure

The figure 1 reveals that the control strain shows a good resistance to ozone because it has all the repair mechanisms. The *lex* A strain is deficient for long patch excision repair (Youngs and Smith 1973) and Type III mechanism (Town *et al.* 1973). According to the curve, this mutant presents an intermediate sensitivity to ozone indicating that either one of these mechanisms could be involved in ozone resistance.

The *pol* A strain used here, is deficient for DNA polymerase I polymerizing function (DeLucia and Cairns 1969) and has lost short patch excision repair (Youngs *et al.* 1974) and Type II mechanism (Town *et al.* 1973). From the curve, this mutant is the most sensitive to the gas among the single mutants. This result suggests DNA polymerase I could play a major role in the repair

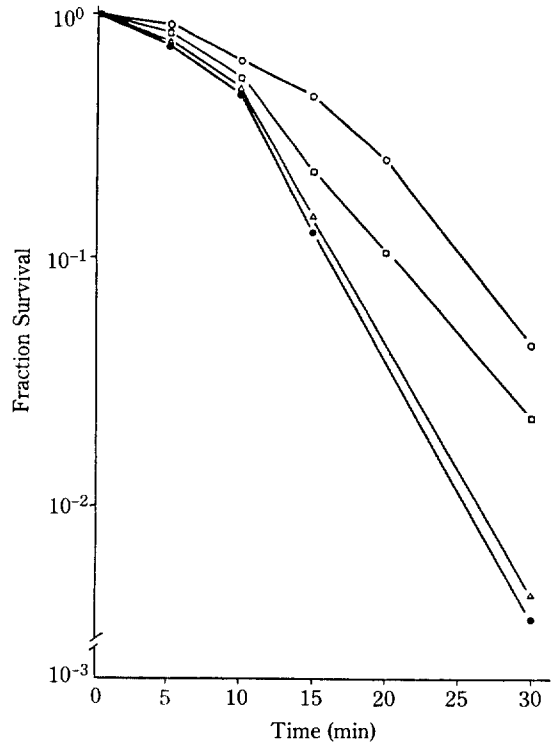

Fig. 1. *Survival curves of E. coli strains DY98 (pol$^+$ lex$^+$) (○), DY99 (lex A) (□), DY100 (pol A) (△) and DY101 (pol A lex A) (●) after exposure to 50 ppm of ozone for 30 minutes.*
The details of ozone treatment are given in Materials and Methods.

of ozone-damaged DNA. The double mutant *pol* A *lex* A shows a sensitivity similar to the *pol* A mutant one. This means the interactions between the mechanisms involving DNA polymerase I and LexA protein would be very secondary.

A *uvr* A mutant is deficient for excision repair completely (Hanawalt *et al.* 1979) and has lost the enzyme activity of the *uvr*ABC endonuclease (Sancar and Rupp 1983). According to the curve presented in figure 2, the ozone sensitivity is very similar to that of the *lex* A mutant. This indicates that the endonuclease could be also involved in ozone resistance. However, previous works have shown that if the ozone treatment was done by another method, such as fumigation (Hamelin and Chung 1974b), the *uvr* A mutant was as resistant as the control strain. Perhaps the experimental conditions might affect the activity of this enzyme.

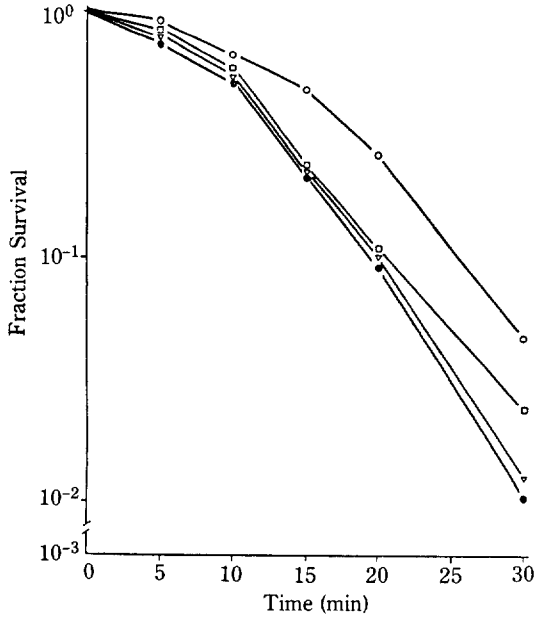

Fig. 2. *Survival curves of E. coli strains DY98 (uvr+ lex+) (○), AB1886 (uvr A) (▽), DY99 (lex A) (□) and DY146 (uvr B lex A) (●) after exposure to 50 ppm of ozone for 30 minutes.*

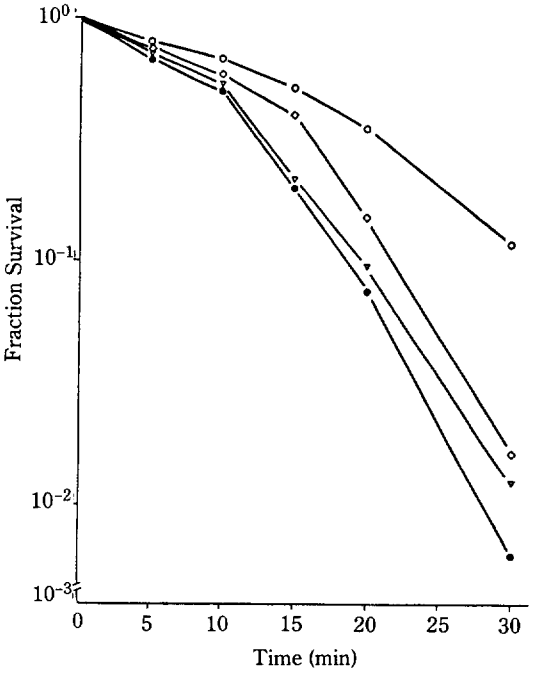

Fig. 3. *Survival curves of E. coli strains AB1157 (uvr+ rec+) (○), AB1886 (uvr A) (▽), AB2463 (rec A) (◇) and AB2480 (uvr A rec A) (●) after exposure to 50 ppm of ozone for 30 minutes.*

The sensitivity of the double mutant *uvr* B *lex* A can be compared to that of the *uvr* A mutant indicating again that the interactions between the repair pathways would be minor.

The *rec* A mutant is deficient for the same mechanisms as a *lex* A mutant, but in addition has lost genetic recombination ability (Moody *et al.* 1973). According to the survival curve (figure 3), the mutant shows an intermediate sensitivity to ozone comparable to the one observed for a *lex* A mutant. This means that the loss of recombination ability does not increase ozone sensitivity. The double mutant *uvr* A *rec* A is slightly more sensitive than either single mutant after 20 minutes of exposure (figure 3) which leads to the same conclusion mentioned above.

For the double mutant *rec* A *lex* A the two genes are this time involved in the same repair pathways. According to the survival curve (figure 4), the ozone sensitivity of this double mutant is expectedly very similar to the sensitivity of the single mutants.

Host-cell reactivation of ozone-exposed T3 phages

It is known that UV irradiated phages such as lambda, T3 and T7 have different plating efficien-

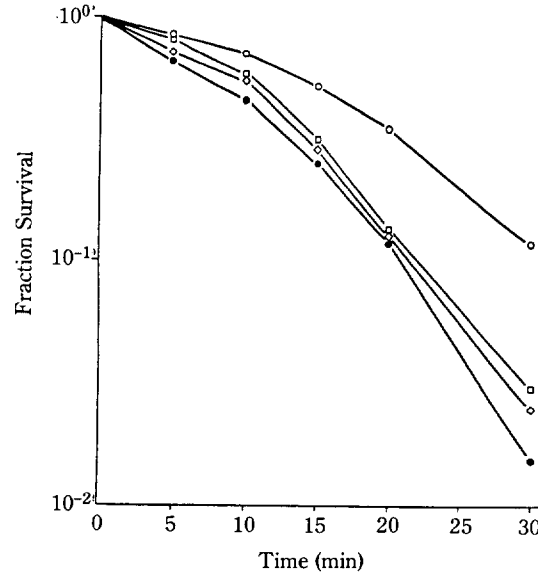

Fig. 4. *Survival curves of E. coli strains AB1157 (lex+ rec+) (○), DM49 (lex A) (□), DM823 (rec A) (◇) and DM824 (lex A rec A) (●) after exposure to 50 ppm of ozone for 30 minutes.*

cy depending on the repair capacity of the host cell in which it multiplies and the process has been called host-cell reactivation (HCR). The capacity of a given strain to reactivate phages, which is measured by the reactivation factor, reflects the resistance of that strain against a DNA damaging agent and can bring precisions about the mechanisms involved in the repair of the damage caused by this agent. We have then determined the host-cell reactivation of ozone-exposed T3 phages for the different bacterial strains used to see if a correlation exists between the survival curves and the capacity to reactivate phages.

The data presented in Table 2 seem to indicate there is a good correlation between ozone resistance and the reactivation efficiency.

The survival fraction for the different strains indicate that T3 phages are rapidly inactivated by the ozone treatment. The control strains have a reactivation factor of 100% because all their repair mechanisms are functional and they can reactivate efficiently the ozone-damaged phages.

Reactivation factors for the different *lex* A and *rec* A mutants are similar and slightly lower than the control one indicating that repair pathways under the control of these two genes would play a minor role in ozone resistance.

A *pol* A mutant reactivates poorly ozone-damaged phages and has the lowest reactivation factor among the single mutants (Table 2). Similar results have been observed for *E. coli* B *pol* A mutants (L'Herault and Chung 1982). These data suggest again that the DNA polymerase I would be important for ozone resistance.

Table 2. *Host-cell reactivation of ozone-treated T3 phages*

Strain	Relevant genotype	Survival fraction	Reactivation factor (%)
DY98		2.12×10^{-3}	100
DY99	(*lex* A)	1.72×10^{-3}	81
DY100	(*pol* A)	9.86×10^{-4}	47
DY101	(*pol* A *lex* A)	8.42×10^{-4}	40
DY146	(*uvr* B *lex* A)	1.54×10^{-3}	73
AB1157		1.15×10^{-3}	100
AB1886	(*uvr* A)	1.31×10^{-3}	114
AB2463	(*rec* A)	9.32×10^{-4}	81
AB2480	(*uvr* A *rec* A)	8.11×10^{-4}	71
DM49	(*lex* A)	9.05×10^{-4}	79
DM823	(*rec* A)	9.50×10^{-4}	83
DM824	(*rec* A *lex* A)	8.85×10^{-4}	77

The reactivation efficiency of the double mutants, if we except the *rec* A *lex* A one, is slightly lower than the one observed for the corresponding single mutants indicating more clearly than the survival curves, that interactions between distinct repair mechanisms are possible but appear very secondary.

However, the situation is very different for a *uvr* A mutant. Eventhough this mutant is sensitive to ozone, as shown by the survival curve (figure 2), it reactivates more efficiently than the control strain ozone-exposed phages. This situation has also been observed for *E. coli* B mutants of this type (L'Herault and Chung 1987).

DISCUSSION

The control strains show a good resistance to ozone (fig. 1 and 3) indicating that the DNA repair mechanisms of these control cells can repair efficiently the damage induced in DNA by this gas. The fact that ozonation of wild-type cells at low doses induces some DNA degradation with practically no decrease in viability supports this conclusion (Hamelin *et al.* 1978).

From both the ozone survival curves reported here and host-cell reactivation data it appears that the DNA polymerase I plays a major role in ozone resistance. The *pol* A mutant is more sensitive to ozone than either *rec* A, *lex* A or *uvr* A single mutants and also more sensitive to the gas than the *uvr* A *rec* A, *uvr* B *lex* A and *rec* A *lex* A double mutants. The *pol* A strain reactivates much less efficiently than any of these mutant strains ozonated T3 phages. Such results are in accordance with those of Hamelin and Chung (1978) who observed that a *pol* A strain is very sensitive to ozone when the ozone exposure was done by another method such as fumigation and also, with the data of L'Herault and Chung (1982) who mentioned that *E. coli* B strains carrying a *pol* A mutation reactivate poorly ozone-damaged T3 phages.

Furthermore, the ozone sensitivity of a *pol* A *lex* A double mutant is very similar to the *pol* A mutant one. From all these data, it turns out then that the repair pathways involving DNA polymerase I, Type II mechanism and/or short patch excision repair, would be the important mechanisms for the repair of ozone-damaged DNA in *E. coli*. The repair pathways under the control of the *rec* A and the *lex* A genes would be also required for ozone resistance, but their role would be a minor one.

The interactions between the distinct repair pathways appear secondary but are possible because all the single mutant strains tested are more sensitive to ozone than the control strain, even if they are deficient for different repair mechanisms. Apart from a *uvr* A strain, they also reactivate less efficiently ozonated T3 phages. Furthermore, the reactivation efficiency of the double mutants is slightly lower than that of their corresponding single mutants. Ozone, being able to produce different kinds of lesions (Gooch *et al.* 1976; Ishizaki *et al.* 1981, 1984; Shinriki *et al.* 1984; Hamelin 1985), enzymatically distinct repair pathways may be required for the repair of different types of lesions or the same kind of lesion could be repaired by different repair mechanisms, a fact well established for pyrimidine dimers after UV irradiation (Hanawalt *et al.* 1979) and DNA single-strand breaks after X-ray irradiation (Town *et al.* 1973).

There is a good correlation between ozone resistance and the capacity to reactivate ozone-treated T3 phages. From the results the strains having a good reactivation efficiency are those showing a strong or at least moderate resistance to the gas. The only exception was observed for the *uvr* A mutant. This mutant is sensitive to ozone according to the method used, but is resistant if the ozone treatment is done by fumigation and it can reactivate very efficiently ozonated phages. We cannot account for this situation at the present moment. L'Herault and Chung (1982) have proposed that the *uvr*ABC endonuclease seems to influence the repair efficiency of the damage caused to DNA by ozone.

The ozone sensitivity observed for these different radiosensitive strains give further evidence that ozone has a radiomimetic activity. This radiomimetic activity has been demonstrated in several ways. Exposures to low concentrations of ozone reduce the dissociation of oxyhemoglobin in human blood (Brinkman and Lamberts 1958) and stimulate the conversion of human and animal erythrocytes in spherocytes in a way similar to that of X-rays (Lamberts *et al.* 1964). Inhalation of this gas induces, like ionizing radiations, chromosomal aberrations in human fibroblasts and leukocytes cultures (Fetner 1962; Gooch *et al.* 1976). The most important similitude between the two agents is that ozone degrades in water to form hydroxyl radicals and other free radicals (Hoigne and Bader 1975; Hamelin and Chung 1976; Grimes *et al.* 1983), which also are responsible for the indirect effects of radiations on cells (Hamelin and Chung 1976). Thus, antioxidant and free radical scavengers, such as reduced glutathione and uric acid which protect against ionizing radiations, also give protection against the toxic effects of ozone (Fairchild 1967; Meadows and Smith 1986).

The radiomimetic activity of ozone as well as the implication of some of the DNA repair mechanisms in ozone resistance suggest the possibility of similitudes between the action mode of ozone and X-rays on DNA. It has been shown that two common types of lesions are produced in DNA by these two agents. For X-rays, it is well known they induce base damage (Cerutti 1975) and mainly the breakage of one or both strands of DNA (Town *et al.* 1973; Ward 1975). Ozone produces the degradation of all nucleobases, especially guanine and thymine, in borate buffer (Ishizaki *et al.* 1981; Meadows and Smith 1986). A treatment of calf thymus DNA with ozone induces a degradation of the base moieties, a loosening of the double-helical structure and the production of single-strand breaks (Shinriki *et al.* 1984). Sawadaishi *et al.* (1985, 1986) reported that ozonolysis of plasmid pBR322 DNA resulted in the degradation of thymine and guanine residues and the formation of single-strand cleavage at specific sites. Ozonation of plasmid pAT153 DNA

caused both single and double-strand breaks (Hamelin 1985).

These two different types of lesions could then be responsible for the observed ozone sensitivity of these mutant strains and especially single-strand breaks for a *pol* A strain. This strain is very sensitive to X-rays because it repairs very poorly induced single-strand breaks (Town *et al.* 1971, 1973), and the lack of repair for this lesion is associated with an extensive DNA degradation (Paterson *et al.* 1971; Glickman *et al.* 1973). Hamelin *et al.* (1977a) observed that such strains degrade their DNA more extensively than a wild-type strain after ozonation. Thus it is very likely, as suggested by them, that unrepaired single-strand breaks are responsible for the low survival and extensive DNA degradation observed in *pol* A strains.

However, the interactions between the distinct repair pathways being very secondary for ozone whereas they are important for X-rays (Town *et al.* 1973) and, moreover, the fact that a *uvr* A mutant is sensitive to ozone (fig. 2) but resistant to X-rays (Kapp and Smith 1970) suggest that some lesions would be specific to ozone. Similar data were observed in *Saccharomyces cerevisiae* (Dubeau and Chung 1979).

적 요

*E. coli*의 방사선 감수성 균주인 lex A, rec A, pol A 등은 DNA 보수기능이 결핍된 균주들로서 야생형 균주에 비하여 오존에 대해서도 감수성이 더욱 민감한 것으로 관찰되었다. 저자들은 *E. coli*의 중복돌연변이체들인 pol A lex A, uvr B lex A, uvr A rec A, rec A lex A 등과 오존에 의하여 손상된 T3 phage간의 숙주 세포 재활성작용과 오존에 대한 저항성을 검증하였다. 실험결과는 DNA polymerase II는 short patch excision 보수 기작에 중요한 효소임을 나타내었다. 서로 다른 DNA 보수 기작의 상호작용은 별 의미가 없으며 오존에 대한 저항성과 오존에 손상받은 T3 phage의 재활성도의 능력은 상호 관련이 있는 것으로 판단되었다.

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