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Induced reactivation of T3 phage in ozone treated strains of *Escherichia coli* B

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오존 처리된 *E. coli* B에서의 T3 파아지의 재활성 유도

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ABSTRACT: The ozone-induced reactivation factor for ozonated or UV-irradiated T3 phage was determined in different bacterial strains of *Escherichia coli* B resistant or sensitive to ozone. Our results suggest that ozone is a weak, if any at all, inducer of the Weigle reactivation, one of the SOS functions. This is in agreement with other studies which have suggested that this agent is probably a weak inducer of the SOS functions.

KEY WORDS: Ozone, Weigle reactivation, T3, SOS functions, *E. coli* B

Weigle reactivation is the increased survival of infective centers of UV-irradiated phage due to certain mutagenic treatments of the host cell before infection (Radman, 1974). Like other inducible phenomena Weigle reactivaton belongs to a class of *rec*A-dependent (Miura and Tomizawa, 1968), *lex*A-dependent (Defais *et al.*, 1971) processes in *Escherichia coli* cells, referred to as the SOS functions (Witkin, 1976), which are expressed following radiation or chemical insult to the cell DNA. Other SOS functions include cell mutagenesis, prophage induction and cell filamentation (Witkin, 1976). Weigle reactivation process was observed in the case of lambda (Weigle, 1953), Pl (Ono and Shimazu, 1966), S13 (Tessman and Ozaki, 1960), 174 (Das Gupta and Poddar, 1975), and T3 phages (Harm, 1963; Kneser, 1968).

Previous results (Kerr and Hart, 1972; Bresler *et al.*, 1978) showed that Weigle reactivation is not specific for UV-irradiated phage and that it occurs also for various types of inactivated phages. A number of different agents besides UV light are able to induce Weigle reactivation such as thymine starvation (Hart and Ellison, 1970) and X-ray irradiation (Martignoni and Haselbacher, 1980).

The aim of this investigation was to determine if ozone could induce the reactivation of ozonated or UV-irradiated T3 phage. This study was based on the facts that: (a) ozone is known to be radiomimetic (Hamelin and Chung, 1976a) and mutagenic (Hamelin and Chung, 1974a; Hamelin *et al.*, 1981; Dubeau and Chung, 1982: L'Hérault and Chung, 1984a), and that it can cause some DNA degradation in different strains of *E. coli* (Hamelin and Chung, 1977a: 1977b; 1981); (b) *lex*A and *rec*A gene products seem to be essential for the recovery of the bacterial cell from the effects of this gas (Hamelin and Chung, 1974b; 1976b; Song and Chung, 1983); and (c) that it has been found to induce filamentation in *lon* strains of *E. coli* (Hamelin and Chung, 1976b). Futhermore, results presented in previous papers (L'Hérault and Chung, 1982; 1984b) showed that ozonated

T3 phage has a different plating efficiency depending on the genetic background of the host cell in which it multiplies.

MATERIALS AND METHODS

The following closely related strains of *Escherichia coli* B were used: B251 (wild type), MQ3060 (*ozr*), MQ3061 (*ozr*), and MQ208 (*uvrA*). The three mutant strains are all derivatives of B251 (Côtes and Chung, 1979; Poliquin *et al.*, 1982). Stocks of T3 phase were prepared by the confluent lysis method with *E. coli* B251 (Adams, 1959). Ozone treatment of T3 phage was performed by the exposure of 10 m*l* of phage water suspensions (10^7 phages/m*l*) to 10 ppm of ozone for 10 minutes while different host cells were exposed to 50 ppm of ozone for 2 to 25 minutes in a similar fashion as described elsewhere (Hamelin and Chung, 1974a; L'Hérault and Chung, 1982). UV-irradiation was carried out with an American Ultraviolet CE-30 germicidal lamp emitting mainly at 254 nm wavelength and at an incident dose rate of 1.52 $Jm^{-2}sec^{-1}$. Water suspensions (2 m*l*) containing 10^6 phages/m*l* were irradiated for 50 seconds (Greenberg, 1967). Host cell reactivation procedures were performed as described in a previous paper (L'Hérault and Chung, 1982) by mixing untreated, ozonated or UV-irradiated T3 phage with ozonated or unozonated log phase of different host cells. The fraction of survivors of ozonated or irradiated T3 phase on different ozonated or unozonated bacterial strains of *E. coli* (S/S°) and the induced reactivation factor (Caillet-Fauquet and Defais, 1972) (phage survival fraction on bacteria ozonated/phage survival fraction on unozonated bacteria) were determined for each different bacterial strain. Control experiments were carried out with clean air instead of ozone.

RESULTS AND DISCUSSION

Dependence of the induced reactivation factor on the ozone treatment administered to the wild type ozone-resistant B251 (Côtes and Chung, 1979; Hamelin and Chung, 1981) host cell is shown in Figure 1. We can see a sharp increase in the reactivation factor of unozonated phage after a short ozone treatment followed by a steady decline with increasing ozone treatment while no such increases are observed in the control experiments. One possible explanation for this higher plating efficiency of T3 phage with ozonated host cell in comparison with unozonated host cell could be a higher efficiency in the adsorption of the phage. It has been reported that ozone can induce damages to cellular membrane (Scott and Lesher, 1963; Pace *et al.*, 1969) which could influence the plating efficiency of T3 phage. Meanwhile, the ozone reactivation factor of ozonated phages increases with increasing treatment time to ozone.

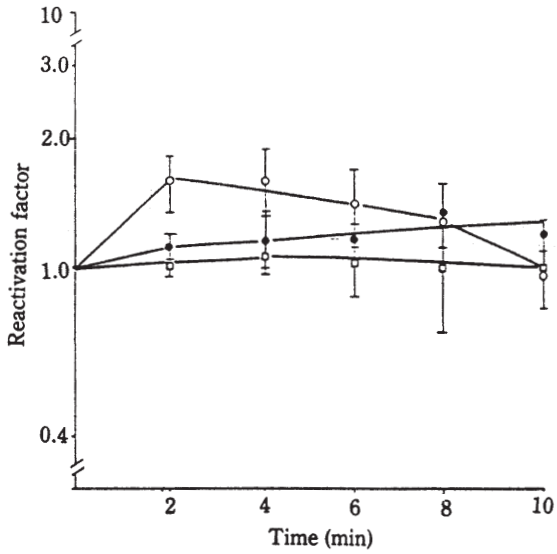

Fig. 1. *Ozone reactivation factor of T3 phage as function of the ozone treatment given before adsorption to the bacterial strain E. coli B251.*
○-○: unozonated phage; ●-●: ozonated pahge; □-□: control experiments with unozonated phage which were carried out with air instead of ozone. Each point is the average of three independent experiments and vertical bars indicate the standard deviation.

In the case of ozone-sensitive mutants MQ3060 and MQ3061 (Côtes and Chung, 1979) (Figure 2 and 3), there is a swift rise in the reactivation factor of ozonated or unozonated phage followed by a sustained decline with increasing treatment time. More important, the later portion of these curves,

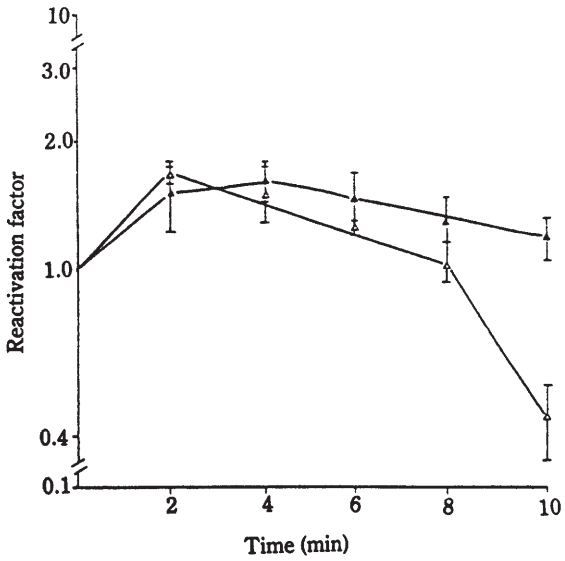

Fig. 2. *Ozone reactivation factor of T3 phage as function of the ozone treatment given before adsorption to the bacterial strain E. coli MQ3060 (ozr).* △-△ :unozonated phage; ▲-▲ :ozonated phage. Each point is the average of three independent experiments and vertical bars indicate the standard deviation.

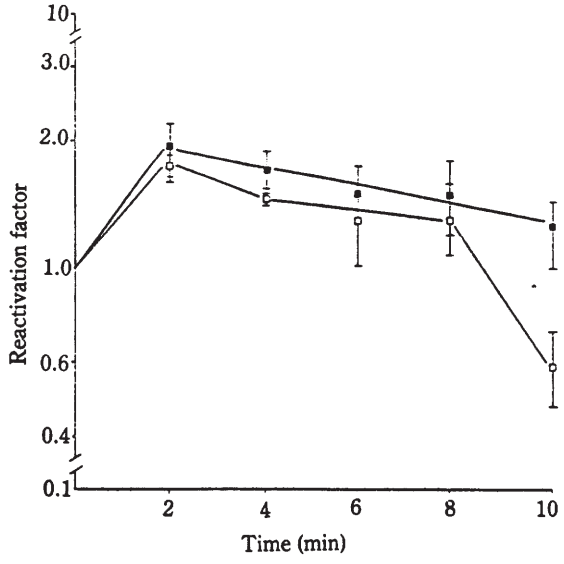

Fig. 3. *Ozone reactivation factor of T3 phage as function of the ozone treatment given before adsorption to the bacterial strain E. coli MQ3061 (ozr).* □-□ : unozonated phage; ■ - ■ : ozonated phage. Each point is the average of three independent experiments and vertical bars indicate the standard deviation.

like with wild type B251 (Figure 1), shows that the capacity of ozonated *ozr* mutants to support growth of unozonated T3 phage decreases much faster than the capacity to support growth of ozonated phage. This could be the first indication that ozone could slightly induce some reactivation process for ozonated phage.

Next we turned to the possible induction of Weigle reactivation (Radman, 1976) by ozone. As previously described in this paper, various physical and chemical agents are able to induce Weigle reactivation of UV-irradiated phage (Hart and Ellison, 1970; Martignoni and Haselbacher, 1980). Some researchers have shown that Weigle reactivation occurs in *uvr*A mutant and that it is induced at lower doses than the wild type (Defais *et al*, 1971). In this series of experiments, the host cell ozonation time was extended to 25 minutes. Once more, as shown in Figure 4, there was an increase in the reactivation factor with the wild type B251 followed by a slower but persistent decline while no such increases were reported for *uvr*A mutant MQ208. Futhermore, the different induc-

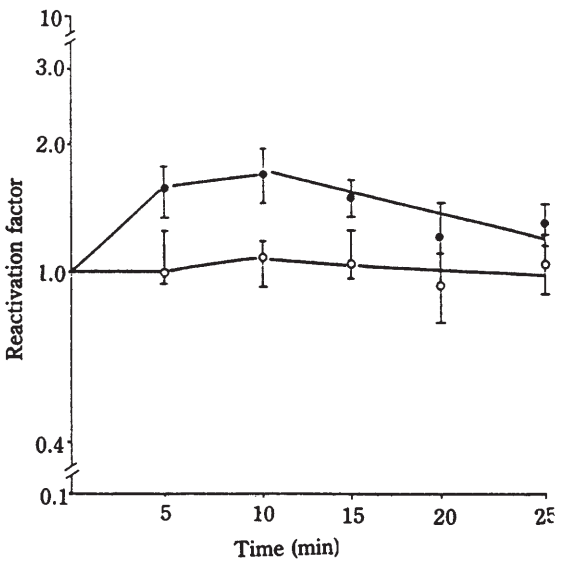

Fig. 4. *Ozone reactivation factor of UV-irradiated T3 phage as function of the ozone treatment given before adsorption to the wild type strain E. coli B251(● - ●)or to the E. coli uvrA mutant MQ208 (○ - ○).*
Each point is the average of three independent experiments and vertical bars indicate the standard deviation.

ed reactivation factors obtained with the wild type B251 vary between 1.3 and 1.7 which is much lower than all the ones obtained with other physical or chemical agents (Kerr and Hart, 1972; Caillet-Fauquet and Defais, 1972; DasGupta and Poddar, 1975; Bresler *et al*, 1978; Martignoni and Haselbacher, 1980).

In conclusion, our results seem to suggest that ozone is probably a weak, if any at all, inducer of the Weigle reactivation. This is in agreement with other studies which have shown that this strong oxidizing gas is a weak inducer of the rec-lex error prone repair mechanism (L'Hérault and Chung, 1984a) and of the prophage induction (L'Hérault and Chung, 1984c), two of the SOS functions.

적 요

오존가스에 감수성이 있는 균주와 저항성을 갖는 여러 종류의 *E. coli* B를 숙주로 사용하여 T3파아지에 자외선 혹은 오존을 처리하므로서 획득된 재활성 요인에 대하여 실험하였다. 그·결과 오존가스는 소위 SOS기능 단위의 하나인 Weigle효과 (=자외선 회복)의 유도원으로서 전연 작용하지 못하거나 작용이 미약함을 알 수 있었는데 이 분야의 다른 보문에서도 일치된 경향을 나타내고 있음을 확인하였다.

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