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EXPERIMENTAL ARTICLES

Inductive Effect of Hypoxanthine–Xanthine Oxidase System on Lambda Prophage[1]

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Abstract—Hypoxanthine–xanthine oxidase (HX–XO) system is a classical system that can generate superoxide anions. The inductive effect of the HX–XO system for lambda prophage was investigated in this study. The results showed that the system can induce lambda prophage from the lysogenic state to lytic growth. The inductive effect was directly proportional to the concentration of HX and XO and inversely related to the time of preliminary incubation of HX with XO.The cell density of the lysogenic bacteria also greatly affected the inductive effect. The maximal PFU number of 2.9×10^4 PFU/ml was recorded at 0.86 mM HX, 1.6×10^{-2} U/ml XO, and a cell density of 10^8 cells/ml. The inductive effect of the HX–XO system was inhibited in the suspensions by glutathione, superoxide dismutase, and catalase. The results provide evidence that free radicals are the primary factors in the induction of lambda prophage in lysogenic bacteria.

Key words: induction, hypoxanthine–xanthine oxidase system, lambda prophage, free radical.

As a superoxide anion generating system, hypoxanthine–xanthine oxidase (HX–XO) is widely used in studies of the physiological function of superoxide radicals [1]. Hypoxanthine is oxidized by xanthine oxidase, and the $O_2^{\bullet -}$ produced may cause reperfusion damage to tissues [2–4]. However, it has not been reported that the HX–XO system can cause changes in the metabolism and reproduction of bacteria.

Bacteriophage lambda DNA integrated into the host chromosome is quite stable in the lysogenic bacterium *Escherichia coli* K12 (λ). However, some physical and chemical factors are known to be able to induce the bacteriophage from the lysogenic state to lytic growth. Ultraviolet light is an effective inducing agent [5, 6]. Tyrrell *et al.* [7] demonstrated that in lysogenic growth, the lytic genes of bacteriophage lambda are switched off by the active CI repressor. When lysogenic cells are irradiated with UV, pyrimidine dimers are formed from adjacent pyrimidines; then, protein RecA is activated and cleaves the CI repressor in the connective region, and the bacteriophage enters lytic growth [8–10]. Our previous study has demonstrated that scavengers of free radicals can efficiently prevent lambda prophage from entering lytic growth when lysogenic cells are irradiated with UV [11].

Our present study is the first to report the inductive effect of the HX–XO system for lambda prophage; it also describes in detail the dependence of the inductive effect on the concentration of HX–XO, on the time of interaction between HX and XO, and on the cell density of the suspension. The results show that free radicals play an important role in the induction of lambda prophage and suggest that the effect of UV irradiation may be mediated by reactive oxygen species formed as a result of irradiation.

MATERIALS AND METHODS

Bacterial Strains and Media

Escherichia coli K12 (λ) and *Escherichia coli* W3001, used as the lysogenic strain and indicator strain, respectively, were obtained from China Center for Type Culture Collection (CCTCC), Wuhan, China. *E. coli* K12 (λ) was grown overnight at 37°C in LB broth containing (per liter) 10 g tryptone, 5 g yeast extract, and 10 g NaCl (pH 7.0). YT broth containing (per liter) 8 g tryptone, 5 g yeast extract, and 5 g NaCl (pH 7.0) and supplemented with 0.5% (w/v) maltose and 10 mM $MgSO_4$ was used for the cultivation of the indicator strain [12].

Induction of Lambda Prophage

An overnight culture of the lysogenic strain *E. coli* K12 (λ) was transferred to fresh LB broth and incubated at 37°C for 4–6 h. The cells were harvested by centrifugation (4000 *g*, 4°C, 15 min), and the pellet was suspended in 0.1 M phosphate buffer (pH 7.0). 0.25 ml of HX (43 mM, Sigma), and 0.25 ml of XO (0.8 U/ml, Sigma) were added to sterilized tubes containing 1.5 ml of the suspension of lysogenic bacteria, and then 2 ml of 2× LB broth was introduced into each tube. Another tube without the HX–XO mixture was used as a con-

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trol. The tubes were kept at 37°C for 3 h. Then, several drops of chloroform were added to the tubes. The tubes were shaken vigorously for 60 s and kept at room temperature for 5 min. After centrifugation (8000 *g*, 4°C, 15 min), the supernatant (the lysate of bacteriophage lambda) was collected for further use [13].

Measurement of the Inductive Effect

An overnight culture of the indicator strain *E. coli* W 3001 was transferred to fresh YT broth containing maltose and $MgSO_4$ and incubated at 37°C for 4–6 h. 0.1 ml of the culture and 0.1 ml of the diluted lysate of bacteriophage lambda were introduced into a sterile tube. The tubes were incubated in a water bath at 37°C for 20 min, after which 5 ml of melted YT agar was added to the tube. After brief mixing, the soft agar was overlaid on the surface of a warmed YT layer in petri dishes. The plates were incubated overnight at 37°C. The inductive effect of the HX–XO system was evaluated from the PFU number revealed on the plates. 70 μl of HX and 70 μl of XO were used as positive controls, and 70 μl of distilled water was used as a negative control [13].

Factors Influencing the Inductive Effect of the HX–XO System

To determine the effect of the concentrations of HX and XO, different amounts were mixed in tubes containing 1.5 ml of a suspension of lysogenic bacteria. The inductive efficiency of the system was determined from titers of the phage. Tubes supplemented with 70 μl of HX or XO alone were used as controls.

To determine the effect of the time of interaction of HX and XO, 70 μl of HX and 70 μl of XO were mixed in tubes containing 2 ml of 2× LB broth and incubated for 0.5, 5, 15, 25, or 45 min. Then, 1.5 ml of the suspension of lysogenic bacteria was added to each tube, and the inductive effects of the systems were determined by the methods described above.

The effect of cell density was studied in the cell density range of 10^6–10^{10} cells/ml. 1.5 ml of a suspension of lysogenic bacteria was placed in each tube, and 70 μl of HX, 70 μl of XO, and 2 ml of 2× LB broth were added to each tube. The inductive effects of the systems were determined as described above.

Inhibitory Effect of Scavengers of Free Radicals

Superoxide dismutase (SOD, Sigma, 500 U/ml), catalase (Sigma, 20000 U/ml) and glutathione (GSH, Shanghai Obo) were used as scavengers of free radicals. The scavengers were mixed with a suspension of lysogenic cells. 70 μl of HX and 70 μl of XO were added to the tubes containing 2 ml of 2× LB broth. The inhibitory effects were tested by the method described above.

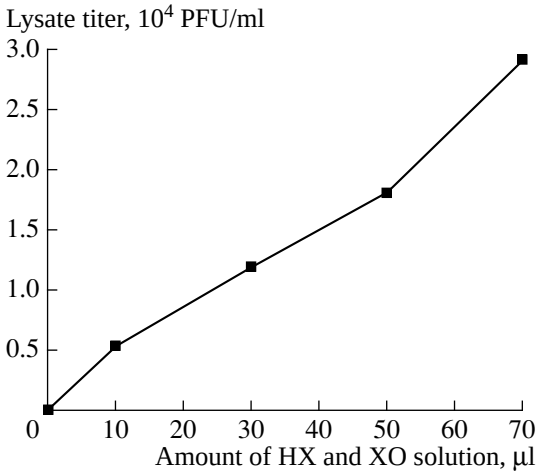

Fig. 1. Dependence of the inductive effect on the amount of HX and XO added.

RESULTS

Induction of Lambda Prophage by the HX–XO System

As shown in Fig. 1, the HX–XO system can efficiently induce lambda prophage to the lytic pathway from the lysogenic state. The inductive effect was closely related to the concentration of HX and XO. With 10 μl of HX and 10 μl of XO (the resulting concentrations of HX and XO in the suspension were 123 μM and 2.3×10^{-3} U/ml, respectively), a bacteriophage titer of 5.3×10^3 PFU/ml was obtained under the conditions of our experiment. The effect was directly proportional to the concentration of HX and XO. The highest titer (2.9×10^4 PFU/ml) was obtained when using 0.86 mM HX and 1.6×10^{-2} U/ml of XO (70 μl of HX and 70 μl of XO). Virtually no plaques appeared on the plates when a solution of HX or XO alone or distilled water was used instead of the HX–XO system.

The Effect of the Time of Interaction between HX and XO

Figure 2 shows the effect of the time of interaction between HX and XO on the induction of lambda prophage. The time of preliminary incubation of HX and XO produced a dramatic effect. When this time was 5 min, a high titer of bacteriophage was recorded (2.6–3.0×10^4 PFU/ml). With the prolongation of the preliminary incubation of HX with XO, the titer rapidly decreased. When this time was 45 min, virtually no plaques appeared on the plate.

Relation between Cell Density and the Inductive Effect

Notable differences were observed between the induction tests with different cell densities of lysogenic bacteria (Fig. 3). The highest titer of the phage (3.0×10^4 PFU/ml) was recorded at a cell density of

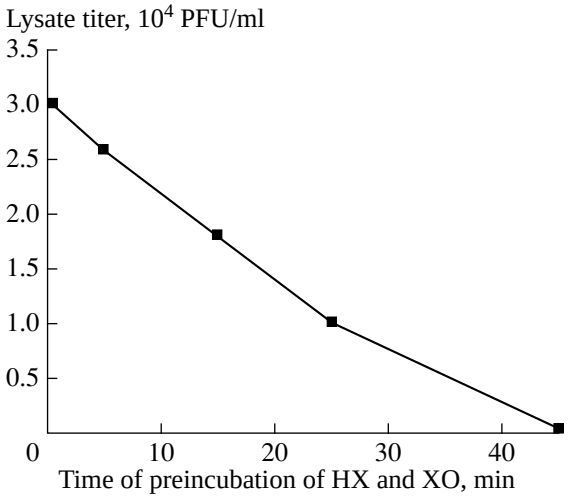

Fig. 2. Dependence of the inductive effect on the time of preincubation of HX with XO.

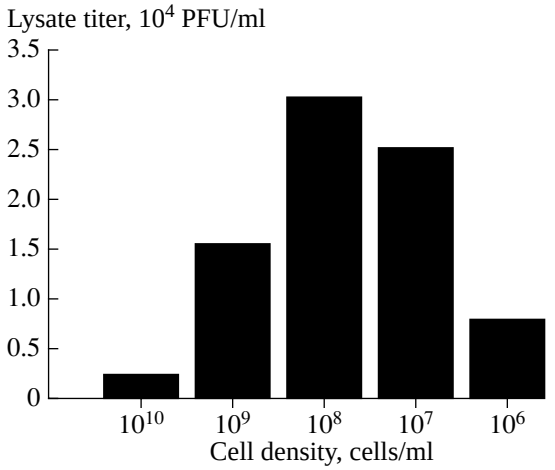

Fig. 3. Relationship between cell density and the titer of phage.

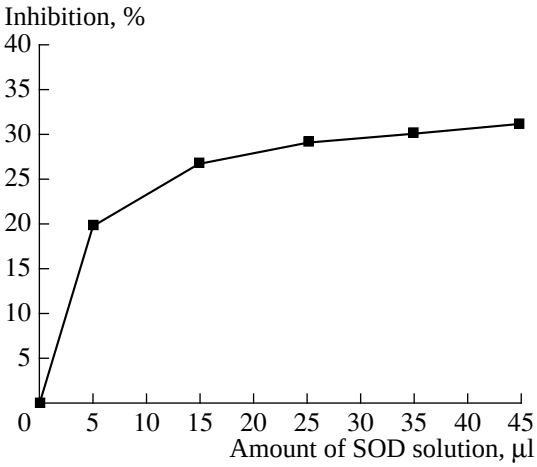

Fig. 4. Inhibition of phage induction by SOD as dependent on SOD concentration.

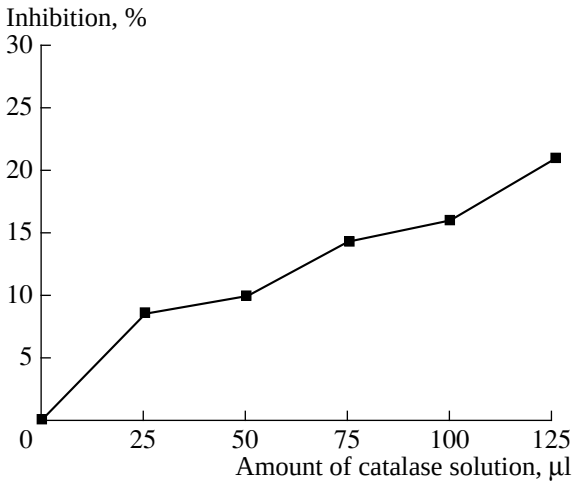

Fig. 5. Inhibition of phage induction by catalase as dependent on catalase concentration.

10^8cells/ml. When the cell density was higher or lower than 10^8 cells/ml, the titers of bacteriophage were lower. The lowest inductive effect was observed at 10^{10}cells/ml.

Inhibitory Effect of Different Scavengers

The inhibitory effect of SOD on the induction of lambda prophage by the HX–XO system is shown in Fig. 4. When the concentration of SOD was 6.4 U/ml, the titer of bacteriophage decreased from 3.5×10^4 to 2.5×10^4 PFU/ml. The inhibitory rate

$$(PFU_{control} - PFU_{scavenger})/PFU_{control} \times 100\%$$

of SOD was about 30%.

Figure 5 shows that catalase also quenched the induction by the HX–XO system, and this also occurred in a dose-dependent fashion, with a maximal inhibitory effect of about 20% at a concentration of 714 U/ml (125 μl). However, when catalase was added to the HX–XO system together with SOD, the inhibitory effects were obviously enhanced. The maximal inhibitory rate was over 75% at a concentration of 3.6 U/ml SOD (25 μl) and 286 U/ml catalase (25 μl) (Fig. 6).

As shown in Fig. 7, GSH could efficiently inhibit the induction of bacteriophage lambda by the HX–XO system in a concentration-dependent fashion, with a maximal inhibitory effect of over 80% at a concentration of 25 mg/ml.

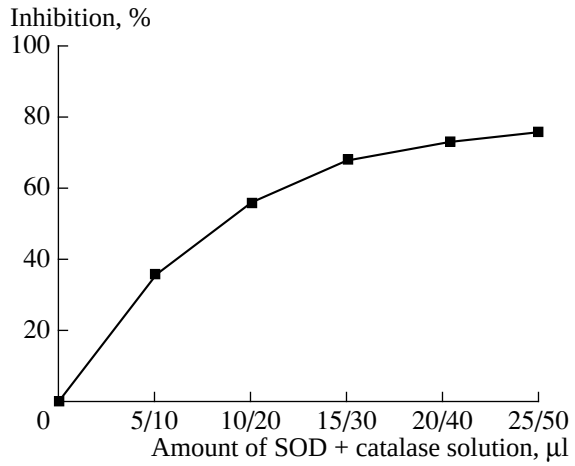

Fig. 6. Inhibition of phage induction by a mixture of catalase and SOD as dependent on its amount.

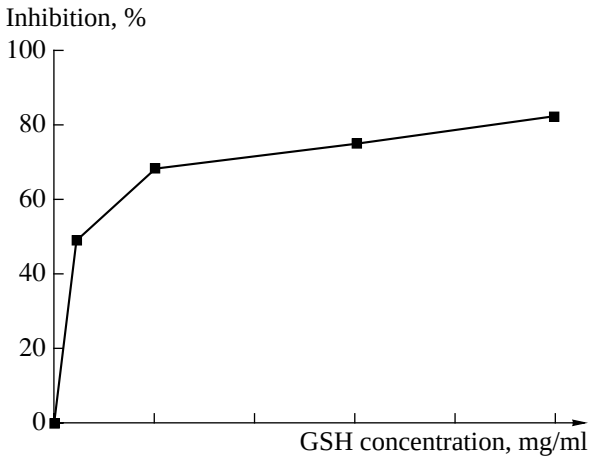

Fig. 7. Inhibition of phage induction by GSH as dependent on GSH concentration.

DISCUSSION

Hypoxanthine–xanthine oxidase, an efficient system for generating free radicals, is often used in the studies of free radicals *in vitro*. However, it has been rarely used in the investigations of metabolic processes. Our results indicate that the HX–XO system can efficiently induce lambda prophage from the lysogenic state to the lytic pathway in the lysogenic bacterium *E. coli* K12 (λ). The inductive effect was not observed when using HX or XO alone under the same conditions. This suggests that the $O_2^{\bullet -}$ formed in the HX–XO system is a significant contributory to the induction of lambda prophage in lysogenic bacteria. This is in agreement with our previous data on the inhibitory effect of ascorbic acid on the UV induction of lambda prophage [14]. Although the free electron can be transferred from $O_2^{\bullet -}$ to other kinds of free radicals, including hydroxide radicals [15], it is evident that $O_2^{\bullet -}$, like ultraviolet irradiation [16], can induce lambda prophage in the lysogenic bacterium *E. coli* (λ). This suggests that free radicals are the primary factors in the induction of lambda prophage from the lysogenic state to lytic growth.

The inductive effect of the HX–XO system depended on the concentration of HX and XO, the time of interaction of HX and XO, and the cell density of the lysogenic bacteria. Namely, the higher the concentrations of HX and XO, the greater the inductive effect. However, we also found that the inductive effect decreased with the prolongation of the interaction of HX with XO. It can be easily understood that the superoxide radicals formed by the HX–XO system quickly disappear with the prolongation of the interaction time of HX and XO because of the instability of the free radicals. The inductive effect was also related to cell density. The maximal PFU numbers were recorded on the test plates when the cell density was 10^8 cells/ml. If the cell density was lower than 10^8 cells/ml, the inductive effect increased with increasing cell density. In contrast, if the density was greater than 10^8 cells/ml, the titer of bacteriophage was reduced with increasing cell density. A possible explanation is that H_2O molecules are necessary for the generation of $O_2^{\bullet -}$ in the system. If cell density is excessive, the volume of H_2O is decreased, and the amount of superoxide anions formed also decreases, bringing about a decrease in the inductive effect, which appears to be strongly dependent on the concentration of free radicals.

SOD can transform $O_2^{\bullet -}$ into O_2 and H_2O_2, and catalase can reduce H_2O_2 to H_2O. In bacterial cells, antioxidants such as SOD, catalase, and GSH can scavenge free radicals [17, 18]. Our results show that SOD or catalase can efficiently inhibit the induction of lambda prophage by the HX–XO system. If both SOD and catalase were present in the system, the inhibitory effect was stronger than produced by either of them alone. H_2O_2, the product formed by O_2 by SOD, can be reduced by catalase, so the mixture of SOD and catalase can scavenge free radicals more completely than either of them alone. We also found that the inhibition rate was very high in the presence of GSH, which is easily understandable since GSH is highly efficient in scavenging free radicals.

Compared with free radicals produced by UV irradiation, free radicals generated by the HX–XO system are lower in concentration and less diverse. Moreover, uric acid, the product of the interaction of HX and XO, can scavenge free radicals as an antioxidant *in vivo* [19, 20]. Therefore, the inductive effect produced on lambda prophage by the HX–XO system is weaker than that produced by ultraviolet irradiation.

In conclusion, the HX–XO system is an effective agent for inducing lambda prophage from lysogenic state to lytic growth, comparable in its efficiency to ultraviolet irradiation. Free radicals play an essential

role in the induction of lambda prophage in lysogenic bacterium *E. coli* and can induce prophage to lysis. This provides helpful evidence for clarifying the mechanism of UV induction of lambda bacteriophage.

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