Note

The Mechanism of Inactivation of Phage ϕ X174 by Ascorbic Acid[†]Akira MURATA, Miyabi HARADA
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To date we have systematically studied the *in vitro* inactivation of phages by ascorbic acid using a wide variety of phages,^{1~3)} and reached the conclusion that ascorbic acid could be effective against all types of phages.⁴⁾ Subsequently, we have studied the mechanism of inactivation of phages by ascorbic acid with several phages of different nucleic acid types.^{3,5~8)}

With respect to single-stranded DNA phages, the mechanism of inactivation by ascorbic acid has been studied with phage δ A, a filamentous phage.^{6,8)} Phage $\phi \times 174$ is a member of another group of phages that contain single-stranded DNA and have symmetrical icosahedral virions.

This paper reports a study on the reaction mechanism involved in the inactivation of phage $\phi \times 174$ by ascorbic acid.

The reaction mixture contained phage $\phi \times 174$ and L-ascorbic acid in Tris-HCl buffer (pH 7.4) and was incubated for 30 min at 37°C.

Bubbling oxygen through the reaction mixture enhanced the inactivation of phage, while bubbling nitrogen fully prevented it. The results indicate that molecular oxygen is required for the inactivation of phages.

Table I shows the effects of the addition of substances to the reaction mixture. Transition metal ions such as Cu^{2+} and Fe^{2+} , and H_2O_2 enhanced the inactivation of phages, while chelating agents such as EDTA and citrate and reducing agents prevented it. The results indicate that traces of transition metal ions are required for the inactivation of phages, and that the inactivation of phages is accompanied by the autoxidation of ascorbic acid.

AET and MEA, general free radical scavengers, prevented the inactivation of phages, suggesting that free radicals generated during the autoxidation of ascorbic acid are involved in the inactivation.

Possible radical species would be the O_2^- , $\text{OH}^\cdot$, and $^1\text{O}_2$, since H_2O_2 , another oxygen radical, at a concentration approximating that theoretically produced by the autoxidation of ascorbic acid, had no direct effect on the phage.

Formate, bromide, iodide, and thiocyanate, specific

scavengers for $\text{OH}^\cdot$, prevented the inactivation of phage, while DABCO, a specific quencher for $^1\text{O}_2$, hardly prevented it. The results suggest the involvement of $\text{OH}^\cdot$, and little involvement of $^1\text{O}_2$, in the inactivation of phages.

As shown in Table II, catalase fully prevented the inactivation of phages, while superoxide dismutase hardly

TABLE I. EFFECTS OF ADDITIONAL SUBSTANCES
ON INACTIVATION OF PHAGE $\phi \times 174$
BY ASCORBIC ACID

Phage $\phi \times 174$ (2×10^7 PFU/ml) was incubated with 2×10^{-4} M L-ascorbic acid and an additional substance in 0.02 M Tris-HCl buffer (pH 7.4) for 30 min at 37°C. Survival of the control (ascorbic acid-free, containing the substance) is taken as 100%.

Addition	Concn. (M)	Survival (%)
None	0	10
Cu^{2+}	1×10^{-6}	0
Fe^{2+}	1×10^{-6}	0 ~ 1
EDTA	1×10^{-5}	90 ~ 100
Sodium citrate	1×10^{-3}	50 ~ 60
H_2O_2	1×10^{-3}	0
L-Cysteine	1×10^{-4}	90 ~ 100
Dithiothreitol	1×10^{-5}	90 ~ 100
Glutathione	1×10^{-5}	90 ~ 100
AET ^a	1×10^{-3}	90 ~ 100
MEA ^b	1×10^{-3}	90 ~ 100
Sodium formate	1×10^{-1}	70 ~ 80
KBr	1×10^{-1}	90 ~ 100
KI	1×10^{-1}	90 ~ 100
KSCN	1×10^{-1}	90 ~ 100
DABCO ^c	1×10^{-2}	10 ~ 20

^a 2-Aminoethylisothiuronium.

^b 2-Mercaptoethylamine.

^c 1,4-Diazabicyclo[2.2.2]octane.

TABLE II. EFFECTS OF ENZYMES ON INACTIVATION
OF PHAGE $\phi \times 174$ BY ASCORBIC ACID

See the legend to Table I, but enzymes were used instead of substances.

Addition	Concn. ($\mu\text{g/ml}$)	Survival (%)
None	0	10
Catalase	100	90 ~ 100
	30	90 ~ 100
	10	60 ~ 70
Superoxide dismutase	100	20 ~ 30
	30	10 ~ 20
	10	10

[†] Virus-inactivating Effect of Ascorbic Acid. Part XIV.

TABLE III. EFFECTS OF OXYGEN RADICALS ON PHAGE $\phi \times 174$

Phage $\phi \times 174$ was added to 0.02 M Tris-HCl buffer (pH 7.4) before each experiment. To generate O_2^- , a weighed amount of solid KO_2 was slowly dissolved in the buffer containing catalase (100 units/ml). To generate 1O_2 , H_2O_2 had been added to the buffer, to which NaOCl was added dropwise. $OH\cdot$ was generated by adding H_2O_2 and $FeSO_4 \cdot 7H_2O$ to the buffer. All reactions were done for 10 min at 37°C.

Generating system	Survival (%)
$KO_2(3 \times 10^{-3} M) \rightarrow O_2^-$	90 ~ 100
$H_2O_2(1 \times 10^{-3} M)$	90 ~ 100
$NaOCl(3 \times 10^{-6} M)$	50 ~ 60
$H_2O_2-NaOCl \rightarrow ^1O_2$	40 ~ 50
$H_2O_2(1 \times 10^{-3} M)$	90 ~ 100
$Fe^{2+}(1 \times 10^{-6} M)$	90 ~ 100
$H_2O_2-Fe^{2+} \rightarrow OH\cdot$	0 ~ 1

prevented it even at high concentrations. The results suggest the little involvement of O_2^- in the inactivation of phages.

The above results, taken together, indicate that $OH\cdot$ might be mainly involved in the inactivation of phages by ascorbic acid. To confirm this, the effects of oxygen radicals generated through chemical reaction on the phage was investigated. As shown in Table III, the phages were

strongly inactivated in an $OH\cdot$ -generating system, but not in an O_2^- -generating system and an 1O_2 -generating system.

It is evident that $OH\cdot$, and not O_2^- and 1O_2 , is the reactive species and that it is directly responsible for the inactivation of phage $\phi \times 174$ by ascorbic acid.

In conclusion, these findings are similar to those obtained with phage δA , another phage that contains single-standard DNA,^{6,8)} and other phages that contains another types of nucleic acids.^{3,5,7,8)}

REFERENCES

- 1) A. Murata, K. Kitagawa and R. Saruno, *Agric. Biol. Chem.*, **35**, 294 (1971).
- 2) A. Murata, K. Kitagawa, H. Inmaru and R. Saruno, *Agric. Biol. Chem.*, **36**, 2597 (1972).
- 3) A. Murata, H. Suenaga, M. Inoue, Y. Tanaka and F. Kato, *Bitamin*, **57**, 515 (1983).
- 4) A. Murata, Proc. 1st Intersec. Cong. Internat. Assoc. Microbiol. Soc., Vol. III, ed. by T. Hasegawa, Science Council of Japan, Tokyo, 1975, p. 432.
- 5) A. Murata and K. Kitagawa, *Agric. Biol. Chem.*, **37**, 1145 (1973).
- 6) A. Murata, R. Oyadorari, T. Ohashi and K. Kitagawa, *J. Nutr. Sci. Vitaminol.*, **21**, 261 (1975).
- 7) A. Murata and M. Uike, *J. Nutr. Sci. Vitaminol.*, **22**, 347 (1976).
- 8) A. Murata, H. Suenaga, S. Hideshima, Y. Tanaka and F. Kato, *Agric. Biol. Chem.*, **50**, 1481 (1986).