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Antiviral Effect of Lithium-Ascorbate Derivatives

– Research note –

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Abstract

The effect of lithium-ascorbate derivatives on viruses was investigated using a wide variety of bacterial viruses (phage). Lithium-ascorbate derivatives exerted an inactivating effect on all phages examined. Lithium-ascorbate derivatives have antiviral effects. The antiviral effect of lithium 2-*o*-octadecyl ascorbate was stronger than that of lithium ascorbate. Even at 10~20 times lower concentration, the lithium 2-*o*-octadecyl ascorbate showed very much similar phage inactivating effect to that of ascorbate and lithium ascorbate.

Key words : lithium ascorbate, lithium 2-*o*-octadecyl ascorbate, phage, antiviral effect

INTRODUCTION

We have studied the virus-inactivating effect of ascorbate by using a wide variety of phages and have shown that oxygen radicals generated during the metalcatalyzed oxydation of ascorbate are involved in inactivating of phages (1-7). Also, the virus-inactivating effect of iron (II)-ascorbate complex was 1,000 ~20,000 times that of ascorbate (8). However, the effect of lithium-ascorbate derivatives on virus has not been studied yet.

We have therefore studied the antiviral effect of lithium-ascorbate derivatives by using a wide variety of bacterial viruses (phage).

MATERIALS AND METHODS

Reagent

Lithium ascorbate and 2-*o*-octadecyl ascorbate were purchased from Takeda Model Co. (Japan). The structure of these compounds is shown in Fig. 1.

Phages

The phages examined are listed in Table 1. The seven phages are various forms and contain different kinds of nucleic acid with different morphologies, and have differences in their host bacteria and other characteristics.

Media and culture conditions

Nutrient broth was used for *Escherichia coli* B, *Escherichia coli* C and *Escherichia coli* K12W3110F$^+$, MRT medium (9) for *Lactobacillus casei* S1, and SSM medium (10) for *Pseudomonas phaseolicola* HB10Y. *Escherichia coli* were incubated with shaking at 37 °C, and *Pseudomonas phaseolicola* HB10Y with sha-

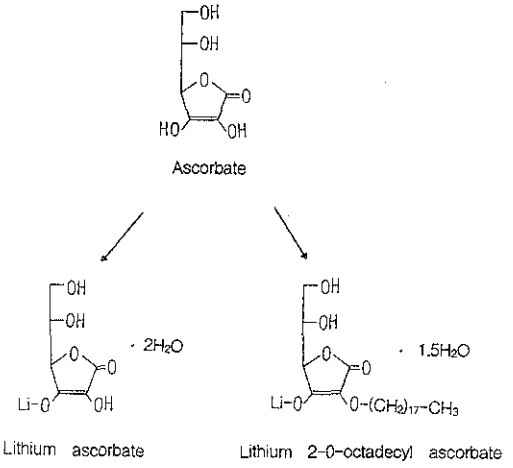

Fig. 1. The structure of lithium-ascorbate derivatives.

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king at 25°C. *Lactobacillus casei* S1 was incubated without shaking at 37°C.

Assay of phages

The survival of phages against lithium-ascorbate derivatives were assayed by the double-agar layer technique (11,12).

Inactivation of phages

Tris-HCl buffer (0.02M, pH 7.4) was used throughout the experiment. A concentrated lithium-ascorbate derivatives solution was freshly prepared prior to each experiment. Phages ($1\sim4\times10^7$PFU/ml) were incubated with lithium-ascorbate derivatives in buffer for 30 min at 37°C. Phage $\phi6$ was incubated for 30min at 25°C because of its relative instability at 37°C. The reaction was conducted in a test tube lightly covered with an aluminium cap and kept vertical. The reaction was stopped by adding a chilled diluent below the effective concentration range of lithium-ascorbate derivatives. The diluent was phosphate buffer (10mM, pH 7.0) containing 1g of NaCl, 0.25g of $MgSO_4 7H_2O$, and 0.03g of gelatin per liter.

Table 1. List of phages examined

Phage	Form and kind of nucleic acid	Morphology	Host bacterial strain
J1 T5	Double-stranded DNA		*Lactobacillus casei* S1 *Escherichia coli* B
T4			*Escherichia coli* B
T3			*Escherichia coli* B
ϕX174	Single-stranded DNA		*Escherichia coli* C
MS2	Single-stranded RNA		*Escherichia coli* K12 W3110 F^+
$\phi6$	Double-stranded RNA		*Pseudomonas phaseolicola* HB10Y

RESULTS AND DISCUSSION

The effects of lithium-ascorbate on the viability of phages are shown in Table 2. Lithium ascorbate inactivated to different extents all seven phages examined. The sensitivities of phages to lithium ascorbate were, from highest to lowest, in the following order : J1, T3, MS2, T5, $\phi\times174$, T4, $\phi6$. When the inactivating potency of lithium ascorbate against each phage was compared with that of ascorbate, no significant difference in potency was seen between these two agents. The effects of lithium 2-*o*-octadecyl ascorbate on the viability of phages are shown in Table 3. Lithium 2-*o*-octadecyl ascorbate inactivated different extents all seven phages examined. The sensitivities of phages to lithium 2-*o*-octadecyl ascorbate were, from highest to lowest, in the following order : T3, $\phi\times174$, MS2, J1, T5, T4, $\phi6$. The phage-inactivating effects of lithium ascorbate was 8~25 times those of ascorbate. These results indicate that lithium-ascorbate derivatives have an inactivating effect on all types of phages.

The reaction mechanism involved in the inactivation of phages by ascorbate reported that free radicals (4,6,8) and OH generated during the auto-oxidation of ascorbate is directly responsible for the inactivation of phage (7).

Thus, there are differences in generation and invol-

Table 2. Effect of lithium-ascorbate on various phages

Concn. (M)	Survival rate (%) of phage						
	J1	T5	T4	T3	$\phi\times174$	MS2	$\phi6$[b]
Lithium-ascorbate[a]							
1×10^{-3}	0	0~1	60~70	0~3	5~10	0~1	80~90
3×10^{-4}	0	10~20	80~100	5~10	15~25	5~20	100
1×10^{-4}	1~5	30~40	100	15~25	40~50	20~40	100
Ascorbate							
1×10^{-3}	0~1	40~50	70~80	0~1	0~1	0~1	40~50

Phages ($1\sim4\times10^7$PFU/ml) were incubated with lithium-ascorbate in 20mM Tris-HCl buffer (pH 7.4) for 30min at 37°C then infected to its own specific host bacteria. The survival rate of the control without lithium-ascorbate is taken as 100%

[a] Concentration as ascorbate (M) [b] Incubated for 20min at 25°C

Table 3. Effect of lithium 2-*o*-octadecyl ascorbate on various phages

Concn. (M)	Survival rate (%) of phage						
	J1	T5	T4	T3	ϕ×174	MS2	ϕ6[b]
Lithium 2-*o*-octadecyl ascorbate[a]							
1×10^{-4}	0~1	0~1	0~1	0	0	0~1	60~80
3×10^{-5}	20~40	30~40	40~60	10~30	20~30	20~40	100
1×10^{-5}	60~80	80~100	100	40~60	40~60	50~70	100
Ascorbate							
1×10^{-3}	0~1	40~50	70~80	0~1	0~1	0~1	40~50

Phages ($1\sim4\times10^{7}$PFU/ml) were incubated with lithium 2-*o*-octadecyl ascorbate in 20mM Tris-HCl buffer (pH 7.4) for 30min at 37°C then infected to its own specific host bacteria. The survival rate of the control without lithium 2-*o*-octadecyl ascorbate is taken as 100%

[a] Concentration as ascorbate (M) [b] Incubated for 20min at 25°C

vement of oxygen radicals in the inactivation of phages between ascorbate and lithium-ascorbate derivatives.

REFERENCES

1. Murata, A. : Virucidal activity of vitamin C, in Proc. 1st. Intersect. Congr. Int. Assoc. Microbial. Soc., Vol. 3, Hasegawa, T. (ed.), Science Council of Japan, Tokyo, p.432 (1975)
2. Murata, A. : Virus-inactivating effect of L-ascorbic acid. *Tanpkkushitsh Kakusan Koso (Protein Nucleic acid Enzyme)*, **20**, 593 (1975)
3. Murata, A., Oyadomari, R., Ohashi, T. and Kitagawa, K. : Mechanism of inactivation of phage δA by ascorbic acid. *J. Nutr. Sci. Vitaminol.*, **21**, 261 (1975)
4. Murata, A. and Uike, M. : Mechanism of inactivation of phage MS2 by ascorbic acid. *J. Nutr. Sci. Vitaminol.*, **22**, [illegible] 376)
5. [illegible] A., Suenaga, H., Inoue, M., Tanaka, Y. and [illegible]ato, F. : Inactivating effect of ascorbic acid on double-stranded RNA phage. *Bitamin (vitamins)*, **57**, 515 (1983)
6. Murata, A. and Kitagawa, K. : Mechanism of in activation of phage J1 by ascorbic acid. *Agric. Biol. Chem.*, **37**, 1145 (1973)
7. Murata, A., Suenaga, H., Hideshima, S., Tanaka, Y. and Kato, F. : Hydroxyl radical as the reactive species in the inactivation of phages by ascorbic acid. *Agric. Biol. Chem.*, **50**, 1481 (1986)
8. Lho, I. H., Morita, K., Myyake, H., Sakamoto, K., Kanda, K., Kato, F. and Murata, A. : Phage-inactivating effect of iron (II)-ascorbate complex. *Biosci. Biotech. Biochem.*, **56**, 720 (1992)
9. Murata, A. : Temperature-sensitive growth of wild-type phage J1 of *Lactobacillus casei*. *Agric. Biol. Chem.*, **35**, 667 (1971)
10. Vidaver, A. K., Koshi, P. K. and Van Etten, J. L. : Phage ϕ6 ; a lipid-containing virus of *Pseudomonas phaseolicola*. *J. Virol.*, **11**, 799 (1973)
11. Adams, M. H. : Bacteriophages. Interscience Publishers, New York, p.443 (1959)
12. Murata, A., Soeda, E. and Saruno, R. : Factors influencing plaque formation by phages of *Lactobacillus acidophilus*. *Nippon Nogekagaku Kaishi*, **43**, 311 (1969)

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아스코르빈산-리듐 유도체의 바이러스에 대한 불활성화 작용

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요 약

아스코르빈산-리듐 유도체의 항바이러스 작용을 검토하기 위하여 여러 종의 박테리아 파아지를 이용하여 이에 대한 불활성화를 조사하였다. 아스코르빈산-리듐 유도체는 조사된 모든 파아지에 대하여 불활성화를 보이므로서 항바이러스 작용이 있음을 알 수 있었다. Lithium 2-o-octadecyl ascorbate의 파아지 불활성화 작용은 lithium ascorbate와 아스코르빈산 보다 10~20배 정도 강하였다. 이러한 결과들은 아스코르빈산-리듐 유도체도 아스코르빈산과 마찬가지로 항바이러스 작용이 있으며 그 정도는 아스코르빈산과 비슷하거나 또는 좀 더 강함을 보여 주었다.