Inactivation of Single-stranded DNA and RNA Phages by Ascorbic Acids and Thiol Reducing Agents

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In previous papers^{1,2)} we found that double-stranded DNA phages (J 1 of *Lactobacillus casei*; T 1, T 2, T 3, T 4, T 5, λ of *Escherichia coli*; and SPOI, PBSI, SPIO, M 2 of *Bacillus subtilis*) were inactivated, in the free state, by L-ascorbic acid, D-araboascorbic acid and thiol reducing agents (glutathione, 2-mercaptoethanol, L-cysteine, dithiothreitol and thioglycollate).

Consecutively, we have found that single-stranded DNA and RNA phages are also inactivated by ascorbic acids and thiol reducing agents.

Single-stranded DNA phages used were ϕ X174 (supplied by Dr. M. Sekiguchi) and δ A (supplied by Dr. T. Sakurai) which were representatives of spherical shape and filamentous shape,³⁾ respectively. RNA phages used were MS 2, GA Q β (supplied by Dr. I. Watanabe) which were representatives of groups I, II and III,^{4,5)} respectively.

Figure and Table show the inactivation rates of single-stranded DNA and RNA phages in various concentrations of the three representative reducing agents, L-ascorbic acid, glutathione (reduced form) and dithiothreitol (reduced form). Similar inactivation was also observed with D-araboascorbic acid, 2-mercaptoethanol, L-cysteine and thioglycollate.

With L-ascorbic acid, D-araboascorbic acid and glutathione, the inactivation rates of the phages were found to be proportional to the

concentration of the agents. Sensitivities of the phages (including some double-stranded DNA phages for comparison) to ascorbic acid and glutathione could be arranged, from the highest to the lowest, in the following order: MS 2, (T 5), Q β , GA, δ A, ϕ X174, (T 4).

TABLE. INACTIVATION BY THIOL REDUCTING AGENTS OF SINGLE-STRANDED DNA AND RNA PHAGES

Phages were incubated with the agent for 30 min. Phage survival of a control without the agent was represented as 100%. Other experimental details were the same as were described in Fig.

Phage	Glutathione		Dithiothreitol	
	Conc. (mM)	Phage survivals (%)	Conc. (mM)	Phage survivals (%)
ϕ X174	3	70	1	88
	7	46	3	63
	10	30	5	85
δ A	3	60	3	74
	7	45	5	51
	10	18	7	72
MS 2	0.1	70	0.1	35
	0.5	32	3	1.4
	3	0.1	5	30
GA	1	63	0.1	74
	5	19	1	10
	10	6.0	7	44
Q β	1	47	0.1	46
	3	8.7	1	0.4
	7	0.8	5	3.0

FIG. Inactivation by L-Ascorbic Acid of Single-stranded DNA and RNA Phages.

Phages (final 2×10^7 PFU/ml) were incubated with L-ascorbic acid in 0.02 M Tris-HCl buffer (pH 7.3) at 37°C . In lots containing high concentrations of the acid, sodium L-ascorbate was used to prevent lowering of pH. Conc. of the acid (final mM) was indicated in the figure. At various time intervals, aliquots were diluted with cold diluent to the order of 10^8 PFU/ml and were plated. The phage assay was based on double-layer technique. As host, *E. coli* C was used for ϕ X174, and *E. coli* K12 W3110 F⁺ for δ A, MS 2, GA and Q β .

----- Control without the acid.

With dithiothreitol, 2-mercaptoethanol, L-cysteine and thioglycollate, the inactivation rates of the phages were maximum at a certain concentration, higher and lower concentrations being less effective. High concentrations of these thiols may protect against inactivation. At the peak concentration, sensitivities of the phages to these thiols could be arranged in the following order: Q β , MS 2, GA, δ A,

ϕ X174.

The patterns of inactivation of single-stranded DNA and RNA phages by reducing agents are similar to those of inactivation of double-stranded DNA phages.

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