ON SOME PROPERTIES OF PHAGE β , A NEW RNA CONTAINING PHAGE

MEIHAN NONOYAMA, ATSUSHI YUKI, and YONOSUKE IKEDA

*Institute of Applied Microbiology,
University of Tokyo, Tokyo*

Received July 10, 1963

Since LOEB and ZINDER (1) isolated a coliphage consisting of RNA and protein, a great deal of attention has been paid to this kind of phages (2, 3, 4) because of their significance in studying the problems pertaining to the multiplication of RNA viruses. This work was undertaken in an attempt to isolate some new RNA phages as well as DNA phages from sewage in Japan. Thus far, the isolated phages were found to be all of RNA type, the selection being made using antiserum of a standard RNA phage MS-2. One of the isolates was phage β , the isolation procedure and some properties of which are described in the present paper. Genetic studies with the phage will be published later.

MATERIALS AND METHODS

Strains. *Escherichia coli* K-12 strain W2252 (Hfr, met⁻ λ^{-}) was used as the indicator strain. RNA phage MS-2 was kindly supplied from Dr. A. J. CLARK of Yale University. The phage multiplies in strain W2252.

Media. As liquid medium, Tryptone broth consisting of Difco Bacto Tryptone 10g, Difco yeast extract 4g, NaCl 8 g, and glucose 1 g in 1,000 ml distilled water was used. The medium with 20 g agar in 1,000 ml Tryptone medium will be referred to as hard agar medium.

Preparation of antiserum of phage MS-2. The antiserum was prepared by injecting about 10^{14} phages in one ml of sterile saline into a rabbit four times at three day intervals. The phage MS-2 was purified by fractionation with ammonium sulfate (40% in final concentration), precipitation with alcohol (50%), and treatment with ribonuclease (RNase, 50 μ g/ml), deoxyribonuclease (DNase, 50 μ g/ml), and trypsin (50 μ g/ml), that were followed by fractional centrifugation. The final preparation of MS-2 thus purified gave a single peak when tested by analytical centrifugation.

Isolation of immunologically related phages. One tenth ml of a sample harvested from the Arakawa Sewage Disposal Plant in Tokyo was plated with the indicator cells (W2252) in 0.8% agar solution onto the agar medium. Plaques which appeared on the plates after 17 hr were stuck by a platinum needle and the phages in them were streaked on soft agar inoculated in advance with the indicator cells. To test whether or not the antiserum could inactivate the isolated phages, the antiserum mentioned above was also streaked

crosswise to the phage line (see Fig. 1). The plates were placed at 37° for 17 hr and the phages which showed no multiplication at the section where the phage line and the antiserum line crossed were judged to be immunologically related with the MS-2.

Fig. 1. Crossreaction test on plate.

Left: crossreacted.

Right: not crossreacted.

Determination of nucleic acids in phages. Two hundred ml of the phage lysate containing about 10^{13} particles were fractionated at 4° by the addition of 40 % ammonium sulfate, and the phage pellets collected by centrifugation at 30,000 rpm were washed twice with 5% perchloric acid (PCA). RNA fraction was prepared by treatment with 0.3% KOH (5) and DNA fraction with 5% hot PCA (5). They were analysed by the orcinol and indole methods (6,7).

RESULTS

Isolation of phages

All of the sewage samples tested gave plaques, and among the 2,000 plaques picked up, 13 were found to be immunologically related with the MS-2. They were subjected to detailed examination. For this purpose, each phage was purified by single plaque isolation and the phage suspension containing about $10^4 \sim 10^5$ particles per ml was treated with the diluted antiserum in saline (0.8 %) containing 0.1 % gelatine. Table 1 shows the number of active phages after treatment with the diluted antiserum, indicating that the degrees of inactivation differ from phage to phage. The inactivation is due to the neutralization of phages with the antiserum. The phages thus selected were named as recorded in Table 1. In contrast, phage T4, a standard DNA phage of *E. coli*, was not affected at all by the treatment as seen in the last column.

Table 1. Neutralization of isolated phages with MS-2 antiserum.
(a)

Phage	Dilution of antiserum					
	2 ¹	2 ⁵	2 ⁷	2 ⁹	2 ¹¹	H ₂ O
MS-2	0	0	0	25	757	10 ⁴
B	0	5	26	99	—	10 ³
C	0	670	1,200	2,400	—	10 ⁴
E	0	23	—	927	1,200	10 ⁴
F	0	293	900	—	—	10 ⁴
I	0	15	75	120	—	10 ³
J	0	2,053	4,428	—	—	10 ⁴
K	63	993	1,080	—	—	10 ⁴
L	0	704	1,732	—	—	10 ⁴
T4	1,400	—	1,500	—	—	1,500

(b)

Phage	Dilution	
	100	H ₂ O
1	40	10 ⁵
3	50	10 ⁵
9	200	10 ⁴
10	1,500	10 ⁵
19	2,200	10 ⁵
β	400	10 ⁵

Antiserum was diluted serially and equal volume of phage suspension was added.

(a) Incubated at 4° for 2 hr. (b) Incubated at 37° for 2 hr.

Nucleic acid in the isolated phages

To know which of the two nucleic acids, DNA or RNA, is contained in the isolated phages, the nucleic acid fractions were analysed by the method described earlier. As seen in Table 2, in which the analytical data are recorded, 11 out of the 12 isolated phages contain large amounts of RNA and negligible amounts of DNA suggesting that the isolated phages are of RNA type. One exception is phage 10 which contains both DNA and RNA. This might be ascribed to the structural binding of a DNA phage with a RNA phage. In contrast, the standard DNA phage T4 gave undetectable amount of RNA and the standard RNA phage MS-2 gave only an insignificant amount of DNA when analysed by the same method.

Host ranges of the isolated phages

Host ranges were studied by the drop test. As shown in Table 3, all of the isolated phages gave a similar host range pattern as the MS-2 although

Table 2. Nucleic acids in isolated phages.

Phage	RNA $\mu\text{g}/200\text{ ml}$	DNA $\mu\text{g}/200\text{ ml}$
B	50	<4
C	30	<4
E	600	<4
F	600	<4
I	300	<4
L	200	<4
J	200	<4
3	500	<4
9	200	<4
10	50	30
19	600	<4
β	300	<4

Table 3. Host range map of isolated phages.

Strain	Episome	Phage													
		MS-2	β	3	9	10	19	B	C	E	F	I	J	K	L
<i>E. coli</i> wild	F ⁺	+	+	+	+	+	+	+	+	+	+	+	+	+	+
58-161	F ⁺	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K-12S	F ⁺	-	-	-	-	-	-	-	-	-	-	-	-	-	-
W3747	F13	+	+	+	+	+	+	+	+	+	+	+	+	+	+
W4520	F-gal	-	-	-	-	-	-	-	-	-	-	-	-	-	-
W3110	F ⁺	+	+	+	+	+	+	+	+	+	+	+	+	+	+
W4397	Hfr	+	+	+	+(?)	+	+	+	+	+	+	+	+	+	+
W2252	Hfr	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3000	Hfr	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P10	Hfr	+	+	+	+	+	+	+	+	+	+	+	+	+	+
W677	F ⁻	-	-	-	-	-	-	-	-	-	-	-	-	-	-
W1177	F ⁻	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C600	F ⁻	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	col E ₁ ⁺	+(?)	+	-	-	+	-	+	-	-	-	+	-	-	-
317	col E ₂ ⁺	-	-	-	-	-	-	-	+	-	-	-	-	-	+
K-235	col K ⁺	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i> B		-	-	-	-	-	-	-	-	-	-	-	-	-	-

exceptions were found in strains carrying colicinogenic factors. As LOEB and ZINDER (1) have pointed out, the isolated phages lysed the Hfr strains of *E. coli* very well, while the lysis of F⁺ strains depended on the strains used.

Fig. 2. Analytical centrifugation pattern of phage- β (left) and MS-2 (right). The conditions employed were: 34,450 rpm at 20° in 0.05 M phosphate buffer, pH 6.8.

Fig. 3. One step growth and premature lysis (phage β).

- (1) intracellular mature phages
- (2) extracellular phages

Fig. 4. β -Galactosidase synthesis in infected cells.

- (1) normal cells
- (2) cells infected with phage F or 10
- (3) cells infected with phage β
- (4) cells infected with T4

Sedimentation patterns of the isolated phages

Purified phages were subjected to ultracentrifugation (Hitachi UCA-1-type). The calculated S values were around 100 in most of the phages, and about 90 and 75 in phage β (Fig. 2). Whether one of these was an aggregation

product or defective particles has not yet been answered. The two peaks shown by phage β were reproduced several times.

Base ratio in RNA of phage β

Phage β was salted out with 40% ammonium sulfate, precipitated with 50% alcohol, treated with RNase, DNase, and trypsin (each 50 μ g/ml), and finally spun down at 30,000 rpm for 60 min. RNA fraction in this preparation was hydrolysed with 1N HCl. The hydrolysates were chromatographed on Toyo Filter Paper No. 51-A using isopropanol-HCl (5.6 N) mixture as the solvent. The mole percents found were : A 23.3, U 28.0, G 21.6, and C 27.2. Minor components were neglected in this analysis.

One step growth experiment and premature lysis

Cells and phage β were mixed in Tryptone broth at a ratio of 1 to 1, and after treatment with the antiserum the infected cells were diluted with Tryptone broth to 10^4 per ml. Shaking the culture gently at 37°, the plaque forming units were determined at intervals. As shown in Fig. 3, the culture began to give rise to new phages at 40 min and an average burst size at 60 min was about 2,000. When the culture was treated with chloroform and lysozyme (100 μ g/ml), phage liberation was observed even at 25 min, indicating that the maturation began at that time. The elimination of glucose from the medium appeared to repress, although slightly, the multiplication.

β -Galactosidase formation in cells infected with phage β

In *E. coli*, cells infected with T-even-phages are unable to synthesize inducible enzymes such as β -galactosidase (8, 9, 10), whereas the cells infected with λ are able to synthesize the enzymes (11). The difference might be due to the difference in degree of host dependency. An interesting result was obtained with cells infected with phage β (Fig. 4). Until 20 min after infection, the infected cells continued to synthesize β -galactosidase at normal rate, while at a time between 20 and 30 min the enzyme formation was suddenly arrested. The slight increase after 20 min might be due to the presence of surviving cells, because it was found in a separate experiment that about 10% of the total cell population could survive even after infection with phage β . In the cases where phage 10 and phage F were employed, the enzyme was produced normally till the time of burst.

DISCUSSION

As described in the introduction, this work was undertaken to isolate RNA phages as well as DNA phages which crossreact with the antiserum of a standard RNA phage MS-2. Although the isolated phages were all of RNA type, the fact does not disprove the existence of immunologically related DNA phages at a low frequency less than one thousandth. At any rate, it was demonstrated that the RNA type coli phages are distributed widely in Japan. Further details concerning phages 10 and β are under investigation.

The isolated RNA phages displayed a similar host range pattern. This might be due to the fact that these phages had been purified several times through the same host W2252. The phages showed different host range patterns at the time of isolation.

It is noteworthy that phage β revealed an unique feature with respect to the β -galactosidase formation. According to a preliminary experiment conducted by the use of methylated albumin column, the synthesis of messenger RNA in the β infected cells was abruptly arrested at about 25 min, while the synthesis of phage RNA was not. The result is consistent with that obtained in the β -galactosidase experiment. The time of mature phage appearance coincides well with the time when the enzyme formation and the messenger RNA formation are arrested. Thus, the process of phage maturation appears to interfere with the expression of host genome. A similar observation has been reported by FENWICK (12) and ZIMMERMAN *et al.* (13) with poliovirus infected cells.

SUMMARY

Thirteen clones of RNA phages acting upon *Escherichia coli* (K-12 strain W2252) were isolated from sewage in Tokyo by the use of antiserum of a standard RNA phage MS-2. The properties of one of the isolates, phage β , were studied in detail. It was found that this phage gives two peaks, about 75 and 90 in the S values, upon analytical centrifugation, and arrests the formation of β -galactosidase at 25 min after infection. The intracellular mature phages begin to appear at the same time.

The authors wish to express their thanks to Dr. A. J. Clark of Yale University for the supply of RNA phage MS-2. This work was partly supported by a Grant in Aid from the Ministry of Education.

REFERENCES

- (1) T. LOEB and N. D. ZINDER: *Proc. Natl. Acad. Sci. U. S. A.* **47**, 282 (1961).
- (2) S. COOPER and N. D. ZINDER: *Virology*, **18**, 405 (1962).
- (3) J. E. DAVIS and R. L. SINSHEIMER: *J. Mol. Biol.*, **6**, 203 (1963).
- (4) R. H. DOI and S. SPIEGELMAN: *Proc. Natl. Acad. Sci. U. S. A.*, **49**, 353 (1963).
- (5) G. SCHMIDT and S. J. THANNHAUSER: *J. Biol. Chem.*, **161**, 83 (1945).
- (6) A. H. BROWN: *Arch. Biochem.*, **11**, 269 (1946).
- (7) G. CERIOTTI: *J. Biol. Chem.*, **214**, 59 (1955).
- (8) J. MONOD and E. WOLLMAN: *Ann. Inst. Pasteur*, **73**, 737 (1947).
- (9) S. BENZER: *Biochem. Biophys. Acta*, **11**, 383 (1953).
- (10) A. P. LEVIN and K. BURTON: *J. Gen. Microbiol.*, **25**, 307 (1961).
- (11) P. STARLINGER: *J. Mol. Biol.*, **6**, 128 (1963).
- (12) M. L. FENWICK: *Virology*, **19**, 241 (1963).
- (13) E. F. ZIMMERMAN, M. H. HEETER and J. E. DARNELL: *ibid.*, **19**, 400 (1963).