SMOOTH SPECIFIC PHAGE ADSORPTION: ENDORHAMNOSIDASE ACTIVITY OF TAIL PARTS OF P22

SHINTARO IWASHITA and SHIRO KANEGASAKI

The Institute of Medical Science, The University of Tokyo, P.O.Takanawa, Tokyo 108

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SUMMARY: The tail parts of phage P22 as well as the phage particles cleave the O-antigen of its host bacterium, Salmonella typhimurium. The cleavage is caused by specific breakage of α-rhamnosyl 1-3 galactose linkages. Thus the tail parts of this phage consist of an enzyme, endorhamnosidase. The enzyme was not detected in nonpermissible strain infected with an amber gene 9 mutant of P22. Head without tail parts gains infectivity only after incubation with the tail parts which carry this enzymatic activity.

Most, if not all, of the smooth specific bacteriophages of the Salmonellae, such as P22 and phage 27 of Bgroup and ϵ^{15} and ϵ^{34} of E group, are known to be similar in size and appearance. These phages consist of a polyhedral head containing DNA, a short neck and a small base plate; the latter is composed of several identical spikes arranged symmetrically around the neck (1). The spikes or the tail parts, consisting of a single protein species, are considered to be responsible for the phage adsorption to its host cells. The receptor sites of these phages are the O-antigen, a component of lipopolysaccharide present on the bacterial surface (2).

Previous studies(3) have shown that phage ϵ^{15} of S. anatum produces an enzyme at its late stage of infection, which recognizes, adsorbs to and cleaves the specific site of its host O-antigen. This enzyme protein is one of the major components of the phage particles and is similar in molecular range to the P22 tail parts. The suggestion was made that the enzyme protein, composing tail parts, is responsible for the phage adsorption.

The assembly process of head and tail parts of P22 has been shown to be temperature dependent and head and tail parts are reassembled in vitro by their incubation under the appropriate condition (4). We could now demonstrate that the purified tail parts of P22 show similar enzymatic activity as the ϵ^{15} enzyme. Both purified phage and its tail parts cleave the specific site of O-antigen of its host.

MATERIALS AND METHODS: Most of the methods and materials used were essentially the same as described previously (3) unless otherwise stated.

Microbiological techniques: Bacterial strains and phages were obtained from Dr. A. Wright. These included the derivatives of S. typhimurium LT-2: DB21 (nonpermissible for

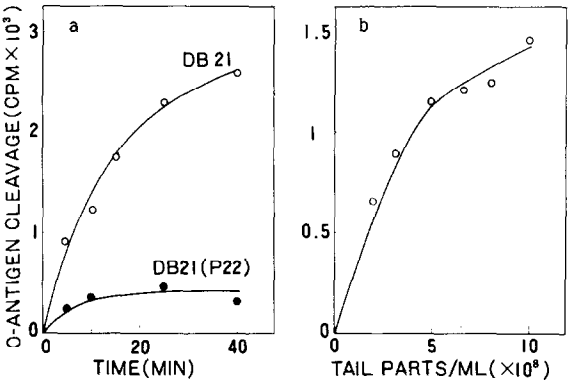

Fig. 1. Cleavage of O-antigen by P22 tail parts.
Indicated amount (b) or 1.5×10^8/ml (a) of purified tail parts in 100 µl of phage 80 buffer were mixed with 10 µl of ^{14}C-labeled cells (6500 cpm (b), 8000 cpm (a)) and incubated at 35° for 10 min or indicated period, respectively. The reaction was terminated by the addition of 12% of acetic acid. The radioactivity retained on membrane filters was measured. The reduced amount of radioactivity was used as a measure of O-antigen cleavage. A sample with no phage tail added was employed as a control.

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phage carring an amber mutation), DB74 (carring an amber suppressor su^+19) and CV2 (lysogens of DB21 carring $P22ts C_2^{29} amN9$), discussed previously by Dr. Botstein (5). The amber mutants of P22 were originally from Dr. Botstein's laboratory (5): amN9, am H101 and amN114 are mutations in tail gene 9, lysis gene 13 and head gene 5, respectively. Bacteria were grown in L-broth. Phages were purified by CsCl density gradient centrifugation and stored in phage 80 buffer.

Preparation of head and tail parts: Tail parts were obtained from strain DB21 infected with $P22amH101amN114C_1^7$ at 35° and purified by ammonium sulfate fractionation and sucrose gradient centrifugation (4). Head particles were prepared by induction of CV2 at high temperature, resulting in a yield of 10^{10} heads/ml. Head particles were purified by banding in CsCl.

Assay of tail parts: The number of tails was calculated from the number of plaque forming units of phages reassembled in vitro at 35° for 1 hour (4).

Assay of O-antigen cleavage: Release of radioactive material by P22 or its tail parts from ^{14}C-labeled S. typhimurium was employed for the assay. The ^{14}C-labeled cells prepared as described previously (3) were stored in 0.01M Tris-HCl buffer (pH 7.4) containing 0.85% NaCl until use.

RESULTS: Phage P22 and its tail parts have the ability to cleave the specific site of the O-antigen of its host as shown below. When the purified P22 particles were incubated with

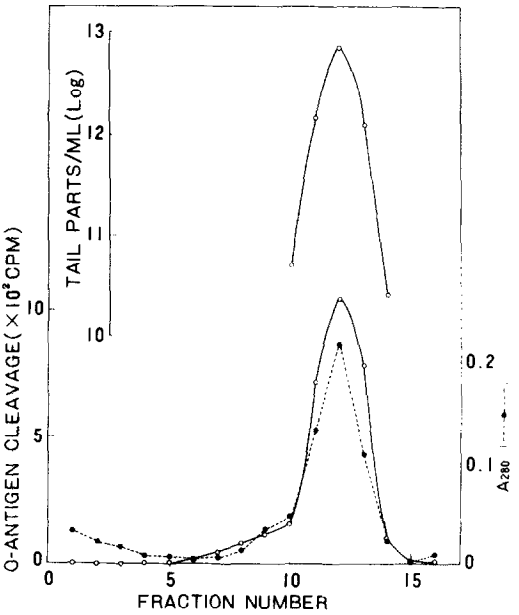

Fig. 2. Sedimentation profile of tail parts and the activity of O-antigen cleavage. Tail parts purified by ammonium sulfate and sucrose gradient centrifugation were layered on a 5-20% sucrose gradient and centrifuged in a Spinco SW 25.1 rotor at 22500 rpm for 26 hours at 15°. Fractions (2 ml each) were collected from the bottom of the tube and assayed for tail parts and O-antigen cleavage.

^{14}C-labeled host cells, S. typhimurium, radioactive material was released, which was characterized as O-antigen. A similar activity was found to be present in purified tail parts obtained from a head defective mutant. The release was proportional to the concentration of tail parts added and increased with time (Fig. 1). A little release was observed if P22 lysogenic cells were used as radioactive substrate. The tail parts on a 5-20% sucrose gradient migrated as a single band, where the releasing activity also recovered (Fig. 2).

The crude lysates were prepared from both permissible and nonpermissible strains of S. typhimurium infected with an amber gene 9 mutant of P22 and assayed for the ability of O-antigen cleavage. The infection was carried out by means of purified tail parts obtained from the head defective mutant. No significant amount of radioactivity was released from ^{14}C-labeled cells incubated with the lysate from the nonpermissible strain (Table 1).

The liberated material from S. typhimurium incubated with tail parts was chromatographed on a Sephadex G-50 column, the results of which is shown in Fig. 3. The three peaks can be distinct but most of the material was of relatively low molecular weight.

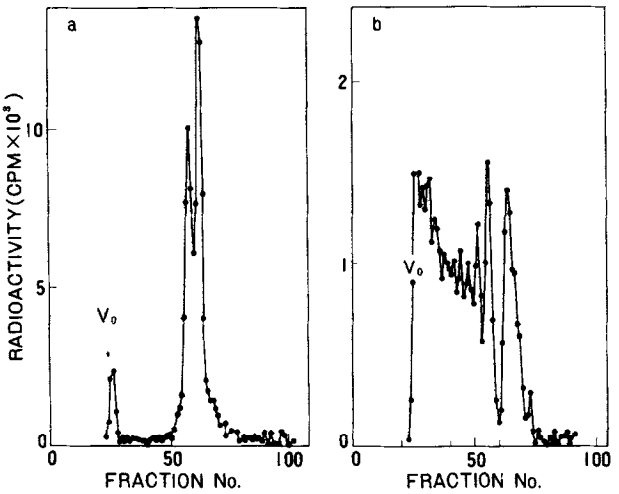

Fig. 3. Analysis of O-antigen cleavage products by Sephadex G-50 chromatography.
(a). Cleavage product wa s produced in a large scale reaction mixture similar to those described in legend of Fig. 1 (200 times scale, 20 min incubation), and separated from cells by filtration through membrane filter. The products was chromatographed on a Sephadex G-50 column (1.8x94 cm: bed volume applox. 230 ml). Fractions were collected (3 ml each) and radioactivity in 0.2 ml aliquots was assayed. Vo indicates the void volume of the column.
(b). Cleavage product obtained from the mixture of P22 particles (3.8×10^9 plaque forming units) and ^{14}C-labeled cells (387000 cpm) in 5 ml was analysed as (a). Incubation for cleavage was carried out at 35° for 5 min.

Analysis of fractions 54-66 by paperchromatography after complete acid hydrolysis indicated that the only radioactive components were the four sugars, mannose, rhamnose, galactose and abequose, showing that P22 tail parts cleaved the O-antigen. When limited number of phage particles were used as enzyme for liberation, the profile of cleavage products on Sephadex G-50 column was somewhat different (Fig. 3-b). The larger materials were obtained and this pattern changed slightly after longer incubation (at 15 min, results not shown), indicating that the tail parts on the phage particles were no longer catalytic after phage bound to the cells in irreversible manner. In this case also, however, the fragments of the O-antigen were the product.

The site of cleavage was further investigated by identifying the end groups generated in the cleavage reaction. Fractions 54-66 from Sephadex G-50 column in Fig. 3-a were combined and reduced with $NaBH_4$. After the removal of borate by Sephadex column, the radioactive material was hydrolyzed with acid and analyzed by paper chromatography. As shown in Fig. 4, a rhamnitol was found to be the only sugar alcohol present in main chain sugars, thus cleavage is caused by specific breakage of α-rhamnosyl 1-3 galactose linkages (see ref. 6).

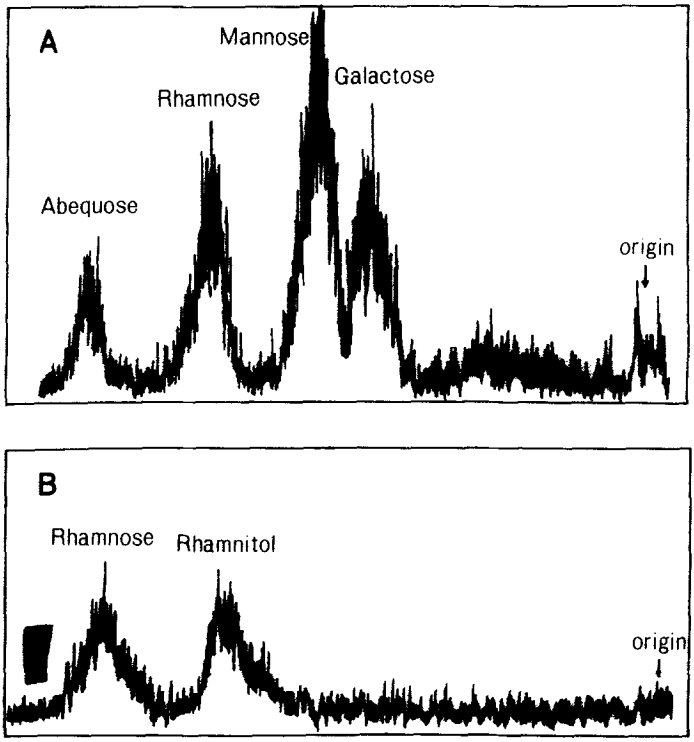

Fig. 4. Paperchromatographic characterization of monosaccharide components of O-antigen cleavage products by P22 tail parts.
The fractions 54-66 in Fig. 3-a were combined, reduced by $NaBH_4$. The radioactive material was purified by Sephadex G-50 column, hydrolyzed with 0.2 N HCl and analysed by paper chromatography. The solvent systems used were 1. BuOH-Pyridine-H_2O (6:4:3) for (A) and 2. BuOH-Pyridine-0.5 M morpholium tetraborate (pH 8.6) (7:5:2) for (B) as detailed in the previous paper (3). The material in the rhamnose region of the first chromatography (A) was rechromatographed by solvent 2, giving separation of rhamnose and rhamnitol (B). Neither mannitol nor galactitol were detected by the similar rechromatography.

DISCUSSION: The tail parts of phage P22 are considered to be essential for its adsorption to the host cells, since the gene 9 defective particles gain the infectivity only after incubation with tail parts. Gene 9 is regarded as the structural gene for the protein of the tail spike (8). Similar phage particles which lack tail parts are formed from wild type phage at relatively higher temperature (7). These particles are also infectious after reconstitution in vitro. We have presented the evidence that each tail part is an enzyme, endorhamnosidase. The enzyme activity was not detected in the lysate of S. typhimurium DB21 (nonpermissible) infected with the gene 9 mutant.

It has been shown that tail parts of P22 themselves bind to the host bacterium and destroy the phage receptor sites (9). We confirmed these results also. Purified tail parts

Table 1. O-antigen cleavage by lysate from P22 amber gene 9 mutant infected cells.

DB74 and DB21 were grown in 30 ml of L-broth to 4×10^8 cells/ml and infected with $P22ts C_2^{29} amN9$, which had been assembled in vitro (at a multiplicity of 5). After 10 min incubation, the infected cells were washed by filtration, resuspended in the original volume of broth and incubated until cell lysis was complete. Cell debris were removed from the lysates by centrifugation and proteins were concentrated by ammonium sulfate precipitation. The O-antigen cleavage activity was measured as described in Fig. 1-a.

Strain	Concentration*	O-antigen released (cpm) 5 min	10 min
DB74 (permissible)	1/3	935	1291
	10/3	1978	1932
DB21 (nonpermissible)	1/3	0	77
	10/3	0	74

* Based on the original concentration

adsorb specifically to its host cells but not to unrelated Salmonella (unpublished data). The specificity must be decided by this enzyme. The cleavage of the host O-antigen may also be essential process in its infection. As shown previously (3), morphorogically similar converting phage e^{15} produces a similar enzyme which adsorbs to and cleaves its host O-antigen.

We have shown that P22 or tail parts cleave O-antigen of S. typhimurium at the specific site, α-rhamnosyl 1-3 galactose linkages. It is interesting to note that e^{15} and the e^{15} enzyme also cleave at α-rhamnosyl 1-3 galactose linkages of O-antigen of S. anatum (3). The whole structures of the O-antigen of both bacteria are quite different in each other (2,6). From the results of conversion study, it is considered that e^{15} recongizes the α-galactosyl 1-6 mannose linkages. The structural change due to conversion by P22 is as follows (10).

S. typhimurium S. typhimurium (P22)

As shown in the present results (Fig. 1-a), the presence of glucose linked 1-6 to galactose inhibits the tail enzyme reaction.

Phage P22 can infect A and D_1 groups of Salmonella as well as B group which includes S. typhimurium (6). These groups of Salmonella share an identical main chain poly-

saccharide and differ essentially only in the stereochemical configuration of the 3,6-dideoxyhexose branches. It is not known whether this fact reflect the broad specificity of the tail enzyme or heterogeneity of O-antigen which may occur in the same strain. Further studies are needed for these points.

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