Mechanism of Adsorption and Eclipse of Bacteriophage ϕ X174

II. Attachment and Eclipse with Isolated *Escherichia coli* Cell Wall Lipopolysaccharide

NINO L. INCARDONA AND LeROY SELVIDGE

*Department of Chemistry and Institute of Molecular Biophysics, The Florida State University,
Tallahassee, Florida 32306*

Received for publication 7 December 1972

A mixture of aqueous phenol, chloroform, and ether extracts the lipopolysaccharides (LPS) from the ϕ X174-sensitive strain, *Escherichia coli* C/1, and resistant strains, C/ ϕ X and K12. Interaction of the C/1 LPS with ϕ X in a starvation buffer containing 10^{-3} M CaCl_2 at 37 C, but not at 15 C, results in a first-order inactivation that is specific for C/1 LPS. After interaction for 60 min at 15 C, followed by centrifugation, 37 and 20% of a ^{14}C - ϕ X preparation are bound to the C/1 and C/ ϕ X LPS pellets, respectively. The results for intact cells are 75 and 10%. Supporting the conclusion that this represents specific attachment of ϕ X to its receptor site in the LPS is the fact that EDTA-borate buffer is required to elute 85% of the ^{14}C - ϕ X from the C/1 LPS, whereas starvation buffer elutes the same amount from C/ ϕ X LPS. Moreover, 95% of the PFU are found in the C/1 LPS pellets as compared with 50% in the resistant strain LPS pellets. When the products of interaction between ϕ X and LPS at 37 C are examined by sucrose density gradients in EDTA-borate, a single 60 to 90S peak is observed in the C/1 sample, and the single peak cosediments with the 120S marker ϕ X in the C/ ϕ X sample. This change in $s_{20,w}$ is very similar to that reported for the eclipse of ϕ X in vivo. If the inactivation at 37 C is carried out on ϕ X-LPS complexes first formed at 15 C, the first-order kinetics are biphasic and nearly identical to that observed for the eclipse kinetics of ϕ X attached to intact cells. Thus, the ϕ X-LPS system is suitable for in vitro studies on the early events in ϕ X infection.

Noncovalent interactions of macromolecules play a central role in the quaternary structure of viruses. This has been aptly demonstrated by the in vitro self-assembly of the simpler viruses from their constituent protein and nucleic acid molecules (1, 6, 12). Even the more complex viruses have many steps in their assembly mechanism that are directed by noncovalent interactions (21). Thus, viruses represent model systems for characterizing the details of these macromolecular interactions and uncovering the molecular mechanism by which they take part in a biological process.

In studying the assembly of viruses, the formation of these interactions among macromolecules becomes important. However, the reciprocal process—their disruption—is of equal importance. It will provide additional data for use in the elucidation of the nature of these interactions. Moreover, the steps by which the viral nucleic acid is delivered into the host cell

represent relatively simple processes for evaluating the role that disruption of these noncovalent interactions plays in reactions within a living organism.

Bacteriophage ϕ X174 has a relatively simple quaternary structure: 12 morphological units, or capsomeres, arranged along the fivefold axes of an icosahedron (7). Each of these, in turn, is composed of at least three different protein subunits of 48,000, 19,000, and 38,000 daltons which occur in the ratio of 5:5:1 (5, 11, 17). The latter two can be selectively removed by urea treatment, leaving an icosahedral particle without the projections, or “spikes,” usually seen in electron micrographs of the native virus (8). Yet it invades its host in a manner common to the more complex tailed bacteriophages. Attachment by the spikes to the receptor sites in the host cell wall (2, 4) is followed by injection of the single-stranded DNA (3, 4, 18). A majority of the viral proteins remain outside the cell (4,

18). These early events in ϕ X174 infection can be separated in vivo into attachment, eclipse (partial injection), and complete penetration of the viral DNA by utilizing the different temperature dependence of their rates and the requirement for active cellular metabolism for the last step (18).

Furthermore, it has been demonstrated that ϕ X alone can undergo an eclipse reaction in vitro that is similar to that observed in vivo (14). However, the rate is at least 10-fold greater in vivo (19), probably reflecting an integrated relationship between the attachment and eclipse steps. Therefore, a further refinement of the in vitro system was sought to reflect more closely the in vivo mechanism. The approach we took was to isolate the receptor site for the virus from the cell wall of its host, *Escherichia coli* C. Previous reports (4, 9) showed that ϕ X adsorbs to isolated cell walls treated with trypsin or lysozyme and to the phenol-insoluble fraction, suggesting the lipopolysaccharide (LPS) nature of the receptor site.

In this report we describe the isolation of a LPS that specifically interacts with the virus. At 15 C the virus attaches to the LPS but still retains its infectivity. At 37 C the interaction causes viral eclipse. This is manifested by a loss in infectivity and a decrease in the sedimentation coefficient of the virus that is essentially identical to that observed with intact cells (18). The striking similarity supports the conclusion that the in vitro system closely reflects the basic mechanisms underlying these early events in the in vivo infection. (A preliminary report of this work has been previously published [Abstr. Biophys. Soc. Meet., 1973].)

MATERIALS AND METHODS

E. coli strains. *E. coli* C/1, the permissive strain for ϕ X174wt, has been previously described (14). For the *am3* mutant of ϕ X, the amber suppressor strain HF4714, kindly provided by R. Sinsheimer, was used. Two resistant strains of *E. coli*, C/ ϕ X and K12, were provided by R. Sinsheimer and J. Stuy, respectively. Both strains are resistant due to the failure of the virus to adsorb at appreciable rates under the usual experimental conditions.

Assay of virus. The diluent and procedure were identical to those used in the previous paper in this series (14).

Starvation buffer. SVB starvation buffer (19) contained 5 g of KCl, 1 g of NaCl, 1.2 g of Tris, 0.1 g of MgSO_4 , and 1.0 ml of 1 M CaCl_2 per liter of distilled water. The pH was adjusted to 8.1 with HCl prior to autoclaving.

Elution buffer. The EDTA-borate elution buffer (18) was 0.05 M in sodium tetraborate and 6 mM in EDTA and saturated with chloroform.

Radioactively labeled ϕ X174am3. *E. coli* C/1 was grown at 37 C to a cell density of 2×10^8 cells/ml in 25

ml of medium B of Newbold and Sinsheimer (18). This medium was composed of 8.0 g of KCl, 0.5 g of NaCl, 1.1 g of NH_4Cl , 0.2 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.023 g of KH_2PO_4 , 0.8 g of sodium pyruvate, 14.76 g of Tris, 1.0 ml of 1 M CaCl_2 , and 1.0 ml of 0.16 M Na_2SO_4 per liter of distilled water. After autoclaving, 0.2 ml of 10% glucose solution is added to 10 ml of medium B. The culture was infected at a multiplicity of 5 with a stock of ϕ X174am3 prepared as described by Hutchison and Sinsheimer (13). Twenty minutes later 387 μCi of ^{14}C -amino acid hydrolysate (New England Nuclear Corp.) was added, and the culture was aerated for 4 h. After storage overnight in the cold room, the cells were collected by centrifugation and lysed with EDTA-lysozyme as previously described (18). After dialysis against 0.05 M borate, the virus was purified from the lysate by one isopycnic and one sucrose density gradient centrifugation. A typical preparation contained 3.5×10^{10} PFU/ml, 630,000 counts per min per ml, and had a specific infectivity of 25%. These values are comparable to those obtained by others (5, 18).

Isolation of LPS. The aqueous phenol-chloroform-ether extraction procedure of Galanos, Luderity, and Westphal (10) was used with *E. coli* strains C/1, K12, and C/ ϕ X. The yields, however, were always between 0.05 and 0.10% by weight. This is lower than that reported by others (10).

Neutral sugar analysis. The analyses were kindly provided by R. J. Winzler as described previously (16).

Sucrose density gradient analyses. After incubation at 37 C, the LPS- ϕ Xam3 mixture was chilled in an ice bath. Then an appropriate amount of $5 \times$ EDTA-borate elution buffer and a purified preparation of ϕ Xam3cs70, as marker, were added. A total volume of 2.0 ml was layered onto a linear 5 to 20% sucrose gradient made up in the EDTA-borate elution buffer. The tubes were then centrifuged at $75,000 \times g$ for 3 h in an SB-110 rotor of an International Equipment Co. model B-60 preparative ultracentrifuge at 5 C. Fractions were collected from a hole punctured in the bottom of the tubes. In the figures the fractions are numbered in descending order so that the direction of centrifugation is from left to right. The marker virus was located by its absorbance at 260 nm. The radioactivity was determined by adding 0.2-ml samples of each tube to 15 ml of a dioxane-based liquid scintillation mixture and counting on a liquid scintillation counter. Only the correction for background was made.

Kinetics of inactivation and eclipse. Lyophilized LPS was weighed out and resuspended in starvation buffer with a glass homogenizer at a concentration of 0.25 mg/ml. The suspension was then cooled in ice and sonically treated three times for 30 s with a Branson model W140 cell disruptor at an output power of 20 to 25 W. The suspension was chilled in ice for several minutes between each sonic treatment. The same suspension was used for a period of about 1 week with the sonic treatment repeated on each day that it was used. For most inactivation experiments, this LPS stock was diluted 1:15 with the starvation buffer.

After 1.8 ml of diluted LPS was preincubated at the

desired temperature in a constant temperature bath regulated to ± 0.03 C, or better, the reaction was started by adding 0.2 ml of diluted ϕ X stock. The final virus concentration was 10^8 PFU/ml. Mixing was achieved by blowing through the pipette prior to each sampling. For the zero time value, another sample was diluted into 1.8 ml of buffer at 0 C. The reaction was stopped by diluting 1:100 in either the phosphate dilution buffer (14) or the elution buffer at 0 C. Preliminary experiments showed that no inactivation takes place at 37 C when the LPS stock solution is diluted 1:1,500 or more.

For the eclipse kinetics, the following modifications of the above procedure were made. A 0.8-ml amount of the stock LPS suspension was preincubated at 15 C for several minutes prior to the addition of 0.2 ml of ϕ X diluted to about 5×10^9 PFU/ml. After 60-min incubation with constant stirring, the reaction tube was chilled in an ice bath. ϕ X-LPS complexes were collected by centrifugation at 5 C for 60 min at $27,000 \times g$ in the SS34 rotor of a Sorvall RC-2B centrifuge. The pellets were resuspended with a Vortex mixer in an equal volume of cold starvation buffer. The eclipse reaction was initiated by diluting 0.1 ml of complexes into 9.9 ml of preincubated starvation buffer at the desired temperature. The reaction was stopped by a 1:50 dilution into cold elution buffer. For the zero time value, a 0.1-ml sample was diluted into cold starvation buffer followed by the dilution into the elution buffer. The method is essentially the same as described by Newbold and Sinsheimer (19).

RESULTS

Characterization of the inactivation of ϕ X with LPS. The assay for the ϕ X receptor site is based on the observation that interaction at 37 C with sensitive cell wall fragments specifically inactivates the virus and renders more than 50% of its DNA sensitive to DNase digestion (3, 4, 9). In starvation buffer at 37 C, the C/1 LPS inactivates ϕ X six times faster than C/ ϕ X LPS, even though the C/ ϕ X LPS is 10 times more concentrated (Fig. 1). With LPS from both K12 and C/ ϕ X, at concentrations equal to that from C/1, there is no detectable inactivation in 1 h. The additional control experiment with virus alone also supports the conclusion that the loss in infectivity is a result of the interaction with the LPS.

If this inactivation is indeed associated with the initial steps in the infection cycle, there are two possible mechanisms. The LPS could attach irreversibly to the virus in such a manner as to block subsequent adsorption to intact cells. Alternatively, the attachment to the LPS could be followed by viral eclipse, thus rendering the particle incapable of subsequent infection of intact cells (18). The fact that no inactivation occurs at 15 C with C/1 LPS (Fig. 1) suggests that the latter is the case. With

FIG. 1. First-order plot of the inactivation of ϕ Xam3 by C/1 LPS in starvation buffer. The ϕ X concentration is 10^7 PFU/ml, and the LPS concentration is 0.017 and 0.18 mg/ml for C/1 and C/ ϕ X, respectively.

intact cells, ϕ X can attach in starvation buffer at 15 C, but the eclipse step requires incubation at 37 C (18).

To distinguish between these two possibilities, we examined the sedimentation coefficient of the virus after inactivation with C/1 LPS. Newbold and Sinsheimer (18) showed that EDTA elution of ϕ X from intact cells after viral eclipse at 37 C yields a noninfectious particle with an $s_{20, w}$ between 60 and 90S. Elution after attachment at 15 C gives the native virus which sediments at 120S. (The usual value given is 114S. However, our extensive sedimentation studies [14], using a method for measuring the temperature within the ultracentrifuge cell [15], gave a value of $120 \pm 2S$.) A sample of ^{14}C -labeled ϕ Xam3 (ca. 2×10^8 PFU/ml) was incubated with C/1 LPS (ca. 0.2 mg/ml) in starvation buffer at 37 C for 30 min and then cooled to 0 C in ice. Plaque assays showed that over 95% inactivation had occurred. An appropriate amount of 5 $\times$ EDTA elution buffer was added, followed by the marker virus, and the mixture was analyzed on a sucrose density gradient. A duplicate experiment with C/ ϕ X LPS was also carried out, but in this case the incubation time was 80 min. Figure 2B shows a single major radioactive peak which cosediments with the 120S marker virus in the sample containing C/ ϕ X LPS, and a single, but slightly broader, 80S peak is observed in the C/1 LPS sample (Fig. 2C). Figure 2A is the result of simply mixing the ϕ Xam3 and the C/1 LPS in the presence of the EDTA elution buffer prior to centrifugation. Thus, the small 50 to 60S peak is present in the stock virus preparation. In every repeat experiment with C/1 LPS a single, though slightly heterogeneous, peak was obtained, but the $s_{20, w}$ values ranged between 60 and 90S.

To test for degradation, the fractions containing the 80S-labeled peak and the 120S marker virus were pooled and dialyzed against 0.05 M sodium borate. Solid CsCl was added to a density of 1.40 g/ml, and the sample was centrifuged at $80,000 \times g$ for 15 h in an IEC A-321 angle head rotor. The distribution of ^{14}C and infectivity in the tube are shown in Fig. 3. About 50% of the 80S particles have the same buoyant density as the marker virus, and the remainder are less dense. The latter could be a result of the loss of some DNA or the stable binding of the LPS to the particles.

Attachment of ϕ X to LPS. In the above experiments the assumption was made that ϕ X binds to the LPS and that the EDTA elution buffer dissociates the complex. Preliminary infectivity assays showed that no inactivation

occurs at 37 C if the virus and LPS are mixed in the elution buffer. A more direct answer was sought in a simple adsorption experiment using the radioactively labeled ϕ X. With a sample of ^3H -labeled C/1 LPS, a centrifugation procedure was tested for pelleting the ϕ X-LPS complexes. After 60 min at $27,000 \times g$, less than 10% of the C/1 LPS is found in the supernatant. As for unbound ϕ Xam3, at least 90% is not pelleted by this procedure (Table 1). When the ^{14}C -labeled ϕ Xam3 and cold C/1 LPS were allowed to interact at 15 C for 60 min at the same concentrations as used in the above sucrose density gradient analyses, 37% of the label was found in the pellet. This is about twice as much as that found in the control experiments with either C/ ϕ X or K12 LPS (Table 1). When these experiments were repeated by using infectivity as a measure of attachment, at least $95 \pm 5\%$ of the PFU were found in the C/1 pellets and only $50 \pm 5\%$ in the K12 pellets. As in the case of the radioactively labeled ϕ X particles, nearly twice as much infectious virus becomes specifically attached. Further support for the view that this twofold difference represents specific attachment of the virus to the LPS is the fact that resuspension of the pellets with the EDTA buffer releases five times as much ^{14}C label and PFU from the C/1 LPS as does starvation buffer (Table 1). In the case of the C/ ϕ X or K12, LPS starvation buffer is as effective as the elution buffer.

If the interaction is carried out at 37 C for 30 min, only 20% of the label is found in the pellet. This is about 50% of the value observed at 15 C. It can be seen from Table 1 that intact C/1 cells pellet 25% fewer counts at 37 than at 15 C. Newbold and Sinsheimer (18) showed that the basis for this difference is that some of the ϕ X particles which eclipse at 37 C spontaneously detach from the cells. In the case of the LPS, the lower efficiency of attachment results in the 37 C value being equal to the nonspecific background attachment. Admittedly, the large standard deviations in these experiments make the results in themselves insufficient proof of attachment of ϕ X to the C/1 LPS. However, taken in the context of the other results, they are consistent with this conclusion.

The data in Table 1 do show that the EDTA borate buffer can elute over 50% of the labeled ϕ Xam3 that is attached at 37 C to sedimentable C/1 LPS. Presumably, it should be capable of dissociating the virus from the 10% of the C/1 LPS that is not pelleted by the centrifugation. Moreover, approximately 50% of the labeled ϕ Xam3 has the same buoyant density as the native virus. Therefore, for these virus particles

FIG. 2. Sucrose density gradient analysis of the virus products after interaction with LPS. Labeled ϕ Xam3 at a concentration of 3×10^8 PFU/ml and LPS at a concentration of 0.18 mg/ml were mixed and incubated at 37 C. After an appropriate time period, the mixture was chilled in an ice bath, 5 X EDTA-borate and marker virus were added, and a sample was layered onto a 5 to 20% sucrose gradient made up in the EDTA elution buffer. After 3 h of centrifugation at $75,000 \times g$, the contents of the tubes were fractionated and analyzed. (A) ϕ Xam3 and C/1 LPS mixed in EDTA elution buffer at room temperature. (B) ϕ Xam3 and C/ ϕ X LPS at 37 C for 80 min in starvation buffer. (C) ϕ Xam3 and C/1 LPS at 37 C for 30 min in starvation buffer.

the decrease in $s_{20,w}$ observed after inactivation is not due to bound C/1 LPS, but to a structural change within the virus particles.

Eclipse kinetics of ϕ X bound to LPS. In measuring the eclipse kinetics of ϕ X attached to intact cells, Newbold and Sinsheimer (19) observed a biphasic first-order plot. At 37 C the initial slope was about five times greater than we observe for the LPS inactivation (0.7 vs. 0.15/

min; see Fig. 1 in both reference 19 and this paper). Moreover, the rate constant that we observed for the LPS inactivation at 37 C is essentially identical to the rate constant for adsorption of ϕ X to intact cells measured by Newbold and Sinsheimer (19) and us (unpublished results) in starvation buffer. If the LPS inactivation is a result of the eclipse reaction, then the rate-limiting step, when the inactiva-

FIG. 3. Isopycnic centrifugation in CsCl of the pooled 80S radioactive peak and 120S marker virus peak of Fig. 2. After centrifugation at $80,000 \times g$ for 15 h, fractions were collected from the bottom of the tube and numbered in the reverse order so that density increases from left to right.

tion is carried out by simply mixing the virus and LPS, would be the adsorption of the virus to the LPS. Therefore, we repeated the inactivation kinetics with C/1 LPS at 37 C after initially forming the ϕX -LPS complexes at 15 C. A preliminary experiment showed that 95% of the initial PFU were bound to the LPS pellet after 60 min at 15 C, and only 6% of the bound PFU were eluted when the complexes were resuspended in starvation buffer. Figure 4 shows that the inactivation of the ϕX bound to the LPS is also biphasic and the initial slope is 0.87/min. Included in the figure are the results we ob-

tained with our preparation of $\phi\text{Xam}3$ attached to intact cells. The initial slopes are indistinguishable.

Partial characterization of the LPS. The lyophilized samples of the LPS from the three strains of *E. coli* were analyzed for their neutral sugar content. The results (Table 2) agree qualitatively with the results on other LPS (10). The mutant of *E. coli* C that was selected for resistance to ϕX has a lower galactose content and higher glucose content than the sensitive strain. However, since the resistant strain K12 is not lacking galactose, these differences may not be related to the receptor site structure. When the ^3H -labeled C/1 LPS was centrifuged

FIG. 4. First-order plot of the inactivation of ϕX attached to C/1 LPS and whole cells. ϕX -LPS complexes were made by incubating 4×10^8 PFU of ϕXwt and 0.20 mg of C/1 LPS per ml at 15 C for 60 min, collecting by centrifugation at $27,000 \times g$ for 60 min, and resuspending in an equal volume of starvation buffer at 0 C. The ϕX -whole cell complexes were prepared by incubating 2×10^8 PFU of ϕXwt and 2×10^8 cells per ml for 30 min at 15 C, collecting by centrifugation at $12,000 \times g$ for 10 min, and resuspending in starvation buffer at 0 C. For the inactivation kinetics, the complexes were diluted 1:100 in prewarmed starvation buffer at 36 C. Symbols: $\bullet$, ϕX -C/1 cell; $\circ$, ϕX -C/1 LPS complexes.

TABLE 1. Adsorption of ϕX174 to host cell wall LPS

Sample	Temp (C)	Initial label in pellet ^a (%)	Pellet label (%) eluted with:	
			SVB	EDTA
Virus alone	15 or 37	10 $\pm$ 6 (5) ^b		
C/1 LPS alone		92 $\pm$ 2 (4)		
C/ ϕX or K12 cells	15	10 (2)		
C/1 cells	15	75 $\pm$ 7 (7)		75 ^c
C/1 cells	37	56 $\pm$ 8 (5)		47 ^c
C/ ϕX or K12 LPS	15 or 37	21 $\pm$ 11 (4)	76 $\pm$ 12 (4)	
C/1 LPS	15	37 $\pm$ 15 (8)	17 (2)	83 $\pm$ 11 (5)
C/1 LPS	37	20 $\pm$ 5 (8)	46 $\pm$ 8 (4)	65 $\pm$ 15 (4)

^a For cells, centrifugation was for 10 min; for LPS the time was 60 min.

^b Number of experiments is shown in parentheses. The standard deviation was not calculated for those values with less than three determinations.

^c From Newbold and Sinsheimer (18).

through a 5 to 20% sucrose gradient for 3 h at $75,000 \times g$, the radioactivity was distributed throughout the gradient. Thus, the sonically treated preparation is heterogeneous with respect to its sedimentation properties, a result also typical for other LPS preparations (20).

DISCUSSION

The extraction procedure used in this study was developed for LPS which have a more lipophilic character (10). Although we did not analyze the lipid content of our LPS, the neutral sugar composition strongly supports its LPS nature. More extensive analyses will be required to assess the purity of our preparations. Also, the observation by Brown et al. (4), that chloroform treatment destroys the ability of *E. coli* C cell wall preparations to inactivate ϕ X, is consistent with the capacity of the aqueous phenol-chloroform-ether mixture to extract the receptor site.

The conclusion that the LPS preparations contain the receptor sites for ϕ X is based on the specificity of the inactivation reaction. It is identical to that exhibited by intact cells for attachment of the virus. This same specificity is retained with cell wall preparations, as has been demonstrated by several groups (3, 4, 9) as well as in our own studies (unpublished results). Thus, the *in vivo* specificity has been maintained throughout the various stages of purification.

The mechanism of the inactivation which is consistent with all of the experimental results is that of viral eclipse. (i) The temperature dependence and the change in sedimentation coefficient induced in the virus particles are strikingly similar to those observed in the *in vivo* eclipse reaction (18). Although a large fraction of our altered particles are less dense than native ϕ X, contaminating nucleases in our LPS preparation could be responsible for this difference. Even if it is argued that the infectious particles are selectively degraded to give the less-dense peaks in CsCl, it has been shown previously that only eclipsed ϕ X is DNase sensitive (4, 18). (ii) The adsorption experi-

ments using both radioactivity and infectivity demonstrate that attachment alone, which occurs at 15 C, does not result in inactivation. (iii) The inactivation kinetics for ϕ X first attached to the LPS at 15 C are almost identical to those observed for the *in vivo* eclipse reaction. Moreover, this conclusion would also explain our failure to demonstrate specific inhibition of the attachment of labeled ϕ X to intact C/1 cells at 15 C by prior interaction of the virus at 15 C with C/1 LPS (concentrations of LPS were the same as those used in the inactivation experiments). If attachment of the LPS to the virus were responsible for the inactivation, then the ϕ X-LPS complex would be incapable of attaching to intact C/1 cells. Thus, the only conclusion that explains all of the above observations is that ϕ X can undergo adsorption and eclipse *in vitro* with the isolated C/1 LPS preparations. This relatively simple system should prove useful in elucidating the mechanism of these early events in ϕ X infection.

ACKNOWLEDGMENTS

The technical assistance of Hattie Sloan and Elvie Fornshell was greatly appreciated.

Funds were provided by a contract with the Division of Biology and Medicine, Atomic Energy Commission, and the Florida Division of the American Cancer Society. A National Science Foundation grant provided support for The Florida State University Computer Center.

LITERATURE CITED

1. Bancroft, J. B. 1970. The self-assembly of spherical plant viruses, p. 99-134. In K. M. Smith, M. A. Lauffer, and F. B. Bang (ed.), *Advances in virus research*, vol. 16. Academic Press Inc., New York.
2. Bayer, M. E., and T. W. Starkey. 1972. The adsorption of bacteriophage ϕ X174 and its interaction with *Escherichia coli*; a kinetic and morphological study. *Virology* 49:236-256.
3. Beswick, F. M., and M. R. Lunt. 1972. Adsorption of bacteriophage ϕ X174 to isolated bacterial cell walls. *J. Gen. Virol.* 16:381-390.
4. Brown, D. T., J. M. Mackenzie, and M. Bayer. 1971. Mode of host cell penetration by bacteriophage ϕ X174. *J. Virol.* 7:836-846.
5. Burgess, A. B. 1969. Studies on the proteins of ϕ X174. II. The protein composition of the ϕ X coat. *Proc. Nat. Acad. Sci. U.S.A.* 64:613-617.
6. Butler, P. J. G., and A. Klug. 1971. Assembly of the particle of tobacco mosaic virus from RNA and disks of protein. *Nature N. Biol.* 229:47-50.
7. Casper, D. L. D., and A. Klug. 1962. Physical principles in the construction of regular viruses. *Cold Spring Harbor Symp. Quant. Biol.* 27:1-23.
8. Edgell, M. H., C. A. Hutchison, and R. L. Sinsheimer. 1969. Process of infection with bacteriophage ϕ X174. XXVIII. Removal of the spike proteins from the phage capsid. *J. Mol. Biol.* 42:547-557.
9. Fujimura, R., and P. Kaesberg. 1962. The adsorption of bacteriophage ϕ X174 to its host. *Biophys. J.* 2:433-449.
10. Galanos, C., O. Luderitz, and O. Westphal. 1969. A new method for the extraction of R lipopolysaccharides. *Eur. J. Biochem.* 9:245-249.
11. Godson, G. N. 1971. Characterization and synthesis of

TABLE 2. Neutral sugar analysis of isolated LPS

Sugar	Percent by weight		
	C/1	C/ ϕ X	K12
Mannose	0.8	0	0
Galactose	2.4	0	2.0
Glucose	3.0	4.6	4.8
Inositol	0.6	0.4	1.0
Heptose	4.4	3.8	4.2
Total	11.2	8.8	12.0

- ϕ X174 proteins in ultraviolet-irradiated and unirradiated cells. *J. Mol. Biol.* **57**:541-553.
12. Hohn, T., and B. Hohn. 1970. Structure and assembly of simple RNA bacteriophages. In K. M. Smith, M. A. Lauffer, and F. B. Bang (ed.), *Advances in virus research*, vol. 16. Academic Press Inc., New York.
  13. Hutchison, C. A., and R. L. Sinsheimer. 1966. The process of infection with bacteriophage ϕ X174. X. Mutations in the ϕ X lysis gene. *J. Mol. Biol.* **18**:429-447.
  14. Incardona, N. L., R. Blonski, and W. Feeney. 1972. Mechanism of adsorption and eclipse of bacteriophage ϕ X174. I. In vitro conformational change under conditions of eclipse. *J. Virol.* **9**:96-101.
  15. Incardona, N. L., H. Notarius, and J. B. Flanagan. 1971. Measurement of temperature within the sample cell during sedimentation velocity experiments. *Anal. Biochem.* **40**:267-280.
  16. Lehnhardt, W. F., and R. J. Winzler. 1968. Determination of neutral sugars in glycoproteins by gas-liquid chromatography. *J. Chromatogr.* **34**:471-479.
  17. Mayol, R. F., and R. L. Sinsheimer. 1970. Process of infection with bacteriophage ϕ X174. XXXVI. Measurement of virus-specific proteins during a normal cycle of infection. *J. Virol.* **6**:310-319.
  18. Newbold, J. E., and R. L. Sinsheimer. 1970. Process of infection with bacteriophage ϕ X174. XXXII. Early steps in the infection process: attachment, eclipse and DNA penetration. *J. Mol. Biol.* **49**:49-66.
  19. Newbold, J. E., and R. L. Sinsheimer. 1970. Process of infection with bacteriophage ϕ X174. XXXIV. Kinetics of attachment and eclipse steps of the infection. *J. Virol.* **5**:427-431.
  20. Weiser, M. M., and L. Rothfield. 1968. The reassociation of lipopolysaccharide, phospholipid and transferase enzymes of the bacterial cell envelope. Isolation of binary and ternary complexes. *J. Biol. Chem.* **243**:1320-1328.
  21. Wood, W. B., R. S. Edgar, J. King, I. Lielausis, and M. Henninger. 1968. Bacteriophage assembly. *Fed. Proc.* **27**:1160-1166.