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# Involvement of a Tryptophan Residue in the Assembly of Bacteriophages φ80 and Lambda

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The assembly of infective particles of bacteriophages λ and φ80 from heads and tails was found to be inhibited by L-tryptophan and some of its analogues, most notably tryptamine. Both the rate of assembly and the final yield of phage were inhibited. The amino acid L-phenylalanine had a slight inhibitory effect, whereas all other amino acids found in proteins were ineffective. Evidence was presented to show that the binding of heads to tails was the affected process in the assay for assembly of infective units. The plaque-forming ability of preassembled phage was not affected by these inhibitors. Results of three different types of experiments suggest that inhibition is due to interaction of inhibitors with the head substructure. The assembly reaction is highly dependent on pH, ionic strength, and the presence of detergents.

Infective particles of the related bacteriophages φ80 and λ can be assembled in vitro from heads and tails isolated from lysates of conditional lethal mutants (2, 7, 11). These two bacteriophages exhibit several structural and functional similarities including homology in base sequence (4) and have been shown to recombine in vivo, forming hybrids (5, 10). Furthermore, in the in vitro assembly reaction, the heads of λ have been shown to unite efficiently with the tails of φ80 to form infective particles, whereas the combination of λ tails and φ80 heads occurs to a very limited extent (2, 6).

The assembly of infective units from the two phage components occurs spontaneously in the absence of any other factor (12). Very little is known about the nature of the bonds involved in this interaction except that they are most likely noncovalent, since phage particles can be dissociated by freezing and thawing and by treatment with sodium dodecyl sulfate (Deeb, unpublished observations).

An interesting approach to the study of the types of linkages involved in T-even phage tail assembly was reported by Kozloff et al. (8) which involved the use of amino acids to either dissociate or inhibit the assembly of phage structures. Arginine and some of its analogues were found to dissociate phage tails and interfere with their assembly. This led to the finding that a C-terminal arginine residue on a phage T4D tail-plate protein forms polar bonds with another tail protein during assembly (9). Utilizing the same type of approach, Kanner and Kozloff (7) showed that adsorption of bacteriophage T-2 particles to host cells was inhibited by indole and certain other related compounds. This was attributed to the ability of these compounds to form charge transfer complexes with a tail fiber protein, thereby changing its configuration so that it could not bind to the host cell. Such a configurational change could be the result of competition between exogenous and endogenous indole rings for certain binding sites on tail fibers.

The possible involvement of lysine in binding heads to tails of the thermophilic bacteriophage TP-80 was suggested by the results of Epstein and Campbell (Abstr. Annu. Meet. Amer. Soc. Microbiol., 1971, p. 211), who demonstrated that dissociation of the phage into heads and tails takes place in the presence of the mentioned amino acid.

This paper describes the results of experiments aimed at investigating the types of linkages involved in the assembly of bacteriophages φ80 and λ from heads and tails by utilizing an approach similar to the ones mentioned above. The results point to the possible involvement of a tryptophan residue(s) on a tail protein in the assembly reaction.

## MATERIALS AND METHODS

**Chemicals and media.** Tryptamine, mitomycin C, *N*,*N*-dimethyltryptamine, and indole-3-propionic acid were highest purity products of the Sigma Chemical Co., St. Louis, Missouri. D- and L-tryptophan and L-phenylalanine were obtained from E. Merck, Darmstadt, Germany. Thymidine-6-$^3H$ (28 Ci/mmol) was obtained from the Radio-

chemical Centre, Amersham, England. Biosolv solubilizer BBS-2,2-(4′-t-butylphenyl), 5-(4′-biphenyl)-1, 3, 4-oxidazole (PBD), and 2-(4′-biphenyl)-6-phenyl-benzoxazole (PBBO) were purchased from Beckman Instruments, Inc. Fullerton, Calif. Antibiotic medium III (Penassay broth), nutrient agar, and broth were products of Difco Laboratories, Detroit, Mich. Davis-Mingioli minimal medium was described previously (1).

**Bacterial and phage strains.** The $\phi$80 suppressor-sensitive (*sus*) mutants, the permissive host *Escherichia coli* K-12 W1485 and the nonpermissive host *E. coli* K-12 594 were described before (2). Lambda *sus* mutants L and A 11 were gifts from J. Weigle (11).

**Preparation of head- and tail-containing lysates.** Defective lysates of head and tail donor mutants of $\phi$80 and $\lambda$ were prepared according to the procedure described previously (3). Unless otherwise indicated, the lysates were in 0.02 M, pH 7.2 potassium phosphate buffer—0.05 M KCl $- 2 \times 10^{-4}$ M $MgCl_2$, hereafter referred to as PCM buffer.

**In vitro complementation.** The reaction mixture, unless otherwise indicated, contained 0.1 ml of each of the head- and tail-containing lysates and 0.1 ml of either PCM buffer or a solution of the inhibitor in the same buffer. Incubation was done at 37 C for 60 min. The reaction was stopped by a 100-fold dilution in half-strength Davis-Mingioli minimal medium. Plaque-forming units were estimated by plating on nutrient agar plates by using *E. coli* K-12 W1485 as an indicator as described previously (2).

**Preparation of phage heads labeled with $^3$H-thymidine.** *E. coli* 594 lysogenic for $\phi$80 *sus* 109 was grown at 37 C in Davis-Mingioli minimal medium supplemented with glucose (0.2%) and an amino acid-containing protein hydrolysate (0.1%) to a cell density of approximately $4 \times 10^8$/ml. The culture was induced with mitomycin C (1 $\mu$g/ml), and 10 min later thymidine-*6-$^3$H* was added to a final concentration of 0.2 $\mu$Ci/ml. Incubation was continued for an additional 3 h after which the culture was centrifuged at 5,000 $\times$ *g* for 15 min, and the cells were frozen. Normally induced cells lyse at 3.5 h after induction, so that centrifugation before lysis allows concentration of the phage heads. Preparation of lysates from the frozen cells was the same as described above for nonradioactive heads.

**Assay for binding of $^3$H heads to tails.** Phage heads that have been labeled with thymidine-*6-$^3$H* were tested for binding to tails according to the following procedure. Equal volumes (0.2 ml) of each of the following were mixed and incubated at 37 C for 1.5 h: $^3$H head lysate, inhibitor dissolved in PCM buffer (or buffer alone in case of control), and tail-containing lysate ($\phi$80 *sus* 9). At the end of this incubation period, 0.2 ml of a freshly grown *E. coli* cell suspension was added (grown in Penassay broth to a concentration of $5 \times 10^8$/ml, washed by centrifugation with PCM buffer, and finally suspended in the same buffer to give a concentration of $2.5 \times 10^9$/ml). Incubation was continued for an additional 20 min. The mixtures were then centrifuged at 4,000 $\times$ *g* for 10 min. The sedimented cells and attached phage were washed twice with 5 ml of buffer and then dissolved by incubation with 1 ml of 2 N NaOH at 80 C for 30 min. To the dissolved cells, 2 ml of Beckman Biosolv solubilizer (BBS-2) were added, and the mixtures were gently shaken until clear. Two drops of 4% $SnCl_2$ in 0.1 N HCl were added, and the mixtures were again gently shaken until clear. Finally, 10 ml of toluene-butyl PBD (0.8%)-PBBO (0.05%) were added, and the mixtures were allowed to stand for 20 min before counting in a Beckman LS-100 liquid scintillation spectrometer.

## RESULTS

**Characterization of the in vitro assembly reaction.** The in vitro assembly of infective particles of $\phi$80 from heads and tails was studied with respect to dependence on ionic strength, pH, and divalent cations to determine optimal assay conditions and to obtain information concerning the nature of bonds between the two phage substructures. Dependence of formation of plaque-forming units on ionic strength is shown in Fig. 1. The reaction is highly dependent on ionic strength with a single optimum at a value of 0.08.

The pH dependence curve shows a single, relatively sharp optimum at approximately pH 7.2 (Fig. 2). Activities at pH 8.5 and above, which are not shown in the figure, are less than 1% of optimal. It is noteworthy that irreversible inactivation of wild-type phage particles occurs at pH values of below 5 and above 10.

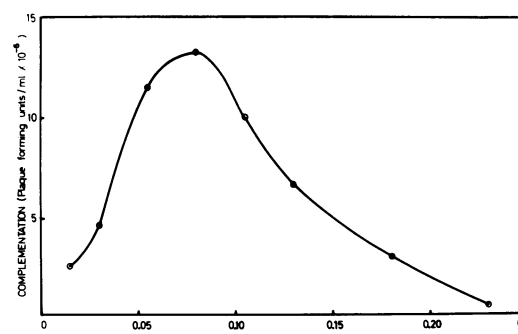


FIG. 1. *Dependence of the rate of assembly of $\phi$80 on ionic strength. Head and tail preparations (sus 109 and 9 were suspended in 0.02 M potassium phosphate buffer (pH 7.2), $2 \times 10^{-4}$ M $MgCl_2$, and an amount of KCl to give the indicated ionic strength values (contributions from proteins in lysates were not included). Equal volumes of the preparations were mixed, and assembly was determined as described in Materials and Methods. The number of heads and tails each was $2 \times 10^7$ per ml of the incubation mixture.*

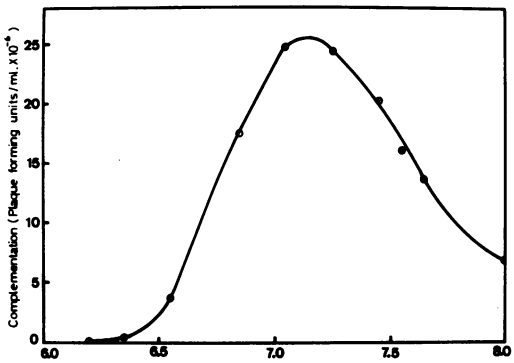


FIG. 2. *Dependence of the rate of assembly of $\phi$80 on pH. Head and tail preparations (sus 109 and 9) were suspended in 0.02 M potassium phosphate buffer adjusted to the indicated pH values. The ionic strength was adjusted to 0.08 with KCl, and $MgCl_2$ was added to a concentration of $2 \times 10^{-4}$ M. Equal volumes of the preparations were mixed, and assembly was estimated as described in Materials and Methods. The number of heads and tails each was $2 \times 10^7$ per ml of incubation mixture.*

Exclusion of divalent cations by chelation with ethylenediaminetetraacetic acid (final concentration 10 mM) resulted in a decrease of approximately 10% in the rate of the assembly reaction. It was found that a $MgCl_2$ concentration of $2 \times 10^{-4}$ M is enough to give optimal activity and was therefore included in all assays.

**Inhibition of in vitro assembly by tryptophan and some of its analogues.** Various amino acids were initially tested for ability to inhibit the in vitro assembly of $\phi$80 and $\lambda$ from heads and tails. At a concentration of as high as 33 mM, L-tryptophan was found to inhibit this assembly by approximately 50%. Of all the other amino acids found in proteins, addition of L-phenylalanine resulted in 15% inhibition. Attention was therefore directed at inhibition by tryptophan by first studying the structural requirements for inhibition.

Several analogues of tryptophan were tested for inhibition of assembly. The results of $\phi$80 (*sus* 9 + 109) and $\lambda$ (*sus* L + A 11) are given in Fig. 3 and 4, respectively. In the former case, L-tryptophan was approximately three times as effective as the D-isomer at the highest concentration tested, whereas in the latter it was twice as effective. More striking however was the finding that tryptamine was about 20 times and 50 times more effective than L-tryptophan in inhibiting $\phi$80 and assembly, respectively. A tryptamine concentration of $4 \times 10^{-4}$ M gave approximately 50% inhibition of assembly. The *N*,*N*-dimethyl derivative of tryptamine was approximately one-tenth as effective in inhibition. Two alkyldiamines, namely hexamethylenediamine and cadaverine, were tested at concentrations of up to 5 mM and found ineffective. The only other striking difference between the $\phi$80 and $\lambda$ systems was that indole-3-propionate was much more effective an inhibitor in the former than in the latter, suggesting the existence of structural differences between the two phage substructures.

When $\phi$80 tails and $\lambda$ heads were used in reactions leading to assembly of hybrid phages, the extent of inhibition by indole-3-propionate was similar to that observed using $\lambda$ heads and tails rather than to that involving $\phi$80 heads and tails. This suggests that the difference between $\phi$80 and $\lambda$ with respect to inhibition by this compound resides in the head.

The question of whether tryptophan and some of its derivatives inhibit the rate of the assembly reaction but not the final amount of phage formed was investigated next. The kinetics of assembly in the presence and absence of inhibitors (Fig. 5) clearly show that both the rate and final

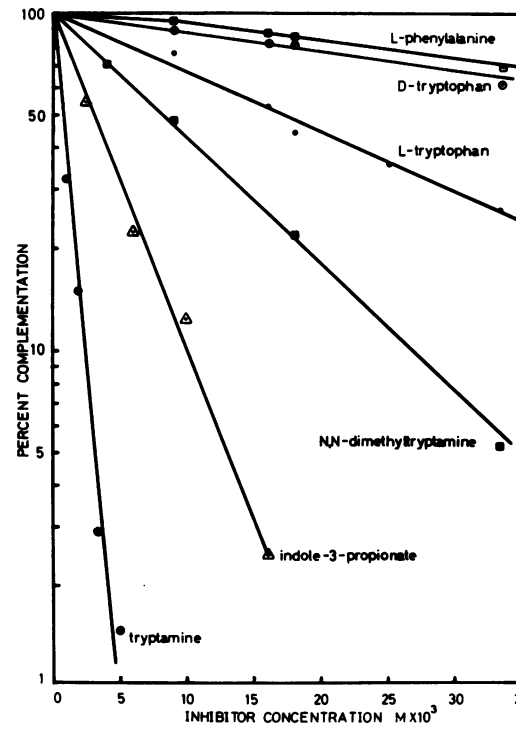


FIG. 3. *Inhibition of the rate of assembly of bacteriophage $\phi$80 by tryptophan and some of its analogues. The conditions of the reaction and assay of complementation were described under Materials and Methods. The number of heads and tails each was $2 \times 10^7$ per ml of reaction mixture.*

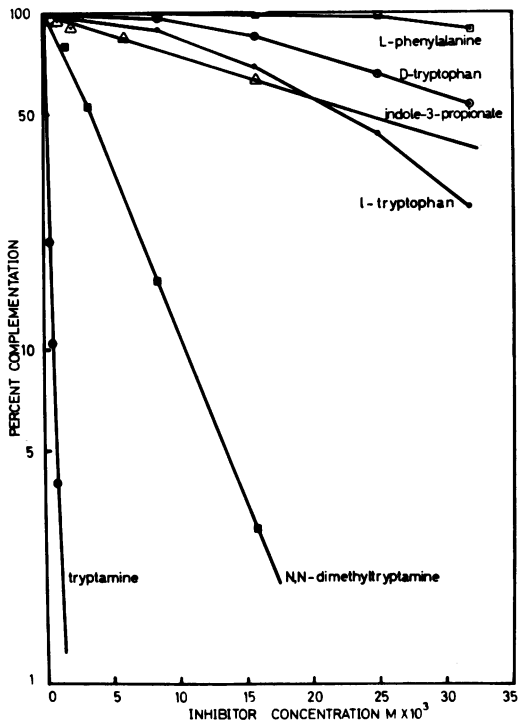


FIG. 4. *Inhibition of the rate of assembly of bacteriophage λ by tryptophan and some of its analogues. The conditions of the reaction and assay of assembly were described in Materials and Methods and in Fig. 3.*

number of phage particles assembled are inhibited.

Inhibition studies using other φ80 head donor mutants (*sus* 107 and 31) and tail donors (*sus* 1 and 8) gave similar results. Therefore, this inhibition is not restricted to only a pair of mutant lysates. Although all of the above mentioned compounds were found to inhibit assembly of phage from heads and tails, they have negligible effects on preassembled viable phage, suggesting that the affected sites are no longer accessible to the added inhibitors.

**Variation of inhibition of assembly with the order of addition of components and with head concentration.** The extent of inhibition of φ80 and λ assembly by tryptamine was determined when either heads or tails were preincubated with the inhibitor. The results using different concentrations of tryptamine are shown in Fig. 6. It is apparent that inhibition is greater when heads were preincubated with tryptamine (about 10 times more effective at a concentration of 3.2 mM in the case of φ80 and at 1 mM in the case of λ). This points to the possibility that phage heads are the target of inhibition.

Further support to the above possibility was provided by results of inhibition studies performed at various head and tail concentrations. The results (Table 1) show that the extent of inhibition increases with decreasing head concentration but is unaffected by decreasing that of tails in the reaction mixture. Taken by themselves, the results of this experiment leave open the possibility that tryptamine competes with heads for a binding site on tails.

**Effect of tryptamine on binding of heads to tails.** The effect of tryptamine on binding the heads to tails rather than on formation of infective phage particles was investigated by using tritium-labeled φ80 heads (formed in the presence of thymidine-6-$^3H$). This would yield information concerning the specific process affected by the inhibitor. The assay, described in detail in the Materials and Methods section, involved incubation of $^3H$ heads with nonradioactive tails in the presence and absence of tryptamine and subsequently binding the complex to *E. coli* cells. The cells with bound phage were isolated and counted. The results obtained are shown in Table 2. It is clear that the binding of heads to tails is the

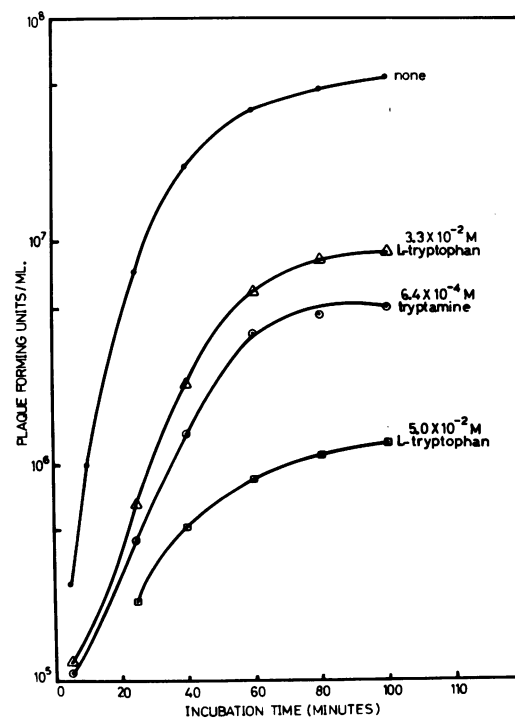


FIG. 5. *Effect of tryptophan and tryptamine on the rate of assembly and the final number of φ80 viable particles. The reaction conditions were described in Materials and Methods. The number of heads and tails each was 2 × 10⁸ per ml of reaction mixture.*

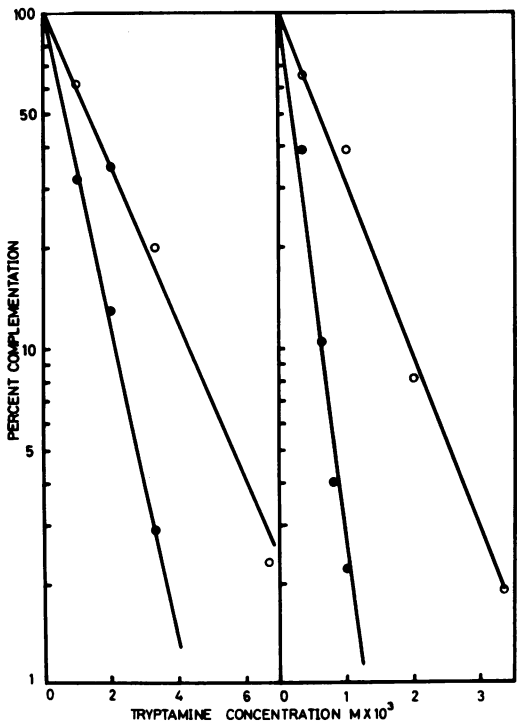


FIG. 6. *Dependence of inhibition of rate of assembly of φ80 and λ on order of addition of components. The panel on the left is for φ80 assembly and that on the right is for λ. Symbols: ●, reactions where the heads (sus 109) were preincubated with tryptamine for 5 min before addition of tails (sus 9); ○, reactions where tails were preincubated with tryptamine prior to the addition of heads. The number of heads and tails was 2 × 10⁷/ml of reaction mixture.*

process that is inhibited by tryptamine. Binding of head-tail complex to cells in this assay was not affected.

## DISCUSSION

The assembly of infective units of bacteriophages φ80 and λ from head and tail substructures was shown to be inhibited by L-tryptophan and some of its analogues. Tryptamine was found to be most effective (approximately 50% inhibition at 1 mM). The *N*,*N*-dimethyl derivative of tryptamine and indole-3-propionate were less effective than tryptamine but more so than troptophan. Alkylamines such as hexamethylenediamine and cadaverine were ineffective. Therefore the structural requirements for optimum inhibition seem to be a tryptophan residue having a free amino group but lacking the negative charge of the carboxyl group. These inhibitors have no effect on the plaque-forming ability of either in vivo or in vitro assembled phage.

Tryptamine was shown to specifically inhibit binding of phage heads to tails and no other phage function. Furthermore, the inhibitor seems to interact with phage heads rather than with tails, rendering them unable to bind to tails. This was evidenced by the dependence of inhibition on head concentration, on preincubation of heads with the inhibitor, and by the observation that inhibition of the union of φ80 tails to λ heads by indole-3-propionate shows the same concentration dependence as inhibition of union of λ tails with

TABLE 1. *Inhibition of rate of assembly by tryptamine as a function of phage φ80 head and tail concentrations*[a]

| Head concn[b] | Tail concn | % Complementation |
|---|---|---|
| 2 × 10⁷ | 2 × 10⁷ | 49 |
| 2 × 10⁷ | 4 × 10⁶ | 43 |
| 2 × 10⁷ | 8 × 10⁵ | 38 |
| 2 × 10⁷ | 2 × 10⁵ | 41 |
| 4 × 10⁶ | 2 × 10⁷ | 28 |
| 8 × 10⁵ | 2 × 10⁷ | 13 |
| 2 × 10⁵ | 2 × 10⁷ | 2 |

[a] Concentration of tryptamine in reaction mixture is equal to 8 × 10⁻⁴ M.

[b] Head and tail concentrations were estimated by mixing with excess tails and heads, respectively, and then assaying for plaque-forming units. The numbers represent plaque-forming units per milliliter of reaction mixture.

TABLE 2. *Effect of tryptamine on binding of ³H heads to tails*[a]

| Reaction mixture[b] | Radioactivity bound to cells (counts/min) |
|---|---|
| 1. Sus 107 ³H (heads) + PCM buffer | 743 |
| 2. Sus 107 ³H (heads) + PCM buffer + Sus 9 (tails) | 4344 |
| 3. Sus 107 ³H (heads) + tryptamine (10⁻³ M) + Sus 9 | 1075 |
| 4. Sus 107 ³H (heads) + tryptamine (5 × 10⁻³ M) + Sus 9 | 870 |
| 5. Sus 107 ³H (heads) + Sus 9 + tryptamine (5 × 10⁻³ M) | 4182 |

[a] Details of assay are given in Materials and Methods.

[b] The components were added in the order in which they are written and incubated for 1.5 h. Assembled phage containing ³H heads were adsorbed to cells and counted after washing.

λ heads, which is different than that for φ80 tails with φ80 heads.

The above observations suggest two possible modes of action of the inhibitors. One is that these inhibitors compete with tryptophan residues (probably *N*-terminal) on tails for certain binding sites on heads necessary to hold the two substructures together. The other possibility is that the inhibitors bind to heads at the site of attachment to tails and induce a conformational change, thereby making heads unable to accept tails. Experiments aimed at differentiating between these possibilities are in progress.

The interaction between phage heads and tails is highly dependent on pH and ionic strength with optima at 7.2 and 0.08, respectively. Plaque-forming ability of complete phage particles varies only slightly between pH 6.5 to 8.5 and ionic strength of 0.04 to 0.18. Coupled with the inhibitory effects of detergents, these results would implicate both ionizable and nonpolar groups in binding of heads to tails.

One might envisage the energy for binding heads to tails to be contributed by ionic bonds and by interactions between the indole ring of tryptophan and other polarizing ring systems or groups. Such interactions could result in high affinity between heads and tails if they occurred in a hydrophobic cavity. Such bonds are known to contribute to the stability of other structures such as hemoglobin and the bacteriophage T2 tail fibers.

## ACKNOWLEDGMENTS

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