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Structural Aberrations in T-Even Bacteriophage
IV. Parameters of Induction and Formation of Lollipops

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Previous results from our laboratory have shown that when a T-even bacteriophage-infected bacterial cell was exposed to L-canavanine followed by an L-arginine chase, a monster phage particle, termed a lollipop, was formed. We now describe certain parameters concerning (i) the induction and (ii) the formation of T4 lollipops. The induction step involves a T4 late function, and can require only a 3-min exposure to L-canavanine. Short pulses of L-canavanine result in the formation of shorter lollipops indicating the presence of a possible "precursor substance" which is influenced by L-canavanine. DNA synthesis is inhibited by L-canavanine but is stimulated 20 to 40 min after the addition of L-arginine. Chloramphenicol prevents both responses indicating a possible protein involvement. The appearance of lollipops and phage was noted only after 25 min after the addition of L-arginine.

Previous results from our laboratory have shown that the L-arginine analogue L-canavanine interferes with the normal growth of T-even bacteriophages (6, 11). The addition of canavanine leads to a marked decrease in progeny phage and apparently affects both viral assembly processes and viral DNA synthesis (7). It has been shown that canavanine leads to an accumulation of polyheads similar to those produced by amber mutants of T4 in gene 20 (15). Both the mutant and induced types of polyheads contain the uncleaved form of the major head protein (P23) which has a molecular weight of about 55,000 (10, 21-23). Upon chasing the canavanine with arginine, a new type of monster phage particle was found which contained DNA and was viable (Fig. 1) (9). The particle, which was termed a lollipop, had an average head length from 3 to 12 times the length of a normal phage head, depending on which T-even bacteriophage was exposed to canavanine. In a lysate containing the longer lollipops, about 30% of the assembled proteins are in lollipop particles. Unlike polyheads, the lollipop was shown to contain the cleaved form of the major head protein (P23*) which has a molecular weight of 45,000 (8, 12, 24). A similar T4 monster particle formed as the result of a mutation in gene 66 has also been described recently (14). Preliminary indications are that gene 66 may be identical with gene 23, the gene coding for the major head protein (A. H. Doermann and F. A. Eiserling, personal communication).

A preliminary study has been made on the effects of canavanine on T4 macromolecular synthesis (7). It was shown that canavanine has little if any effect on net protein or RNA synthesis. However, viral DNA synthesis was reduced about 70 to 80% by canavanine. The gene 66 mutants appear to have a normal pattern of DNA synthesis (A. H. Doermann and F. A. Eiserling, personal communication). The complex relationship between T4 DNA synthesis and phage maturation is not well understood. DNA replication is required for optimal expression of late genes (3, 15, 27) although this effect may depend on the method used to inhibit phage DNA replication (1, 5, 27). Head gene mutations have been shown to interfere with the proper maturation of DNA (18, 19).

The canavanine-induced lollipop presents a unique system with which to study the relationship between DNA replication and phage maturation. We are investigating the role of canavanine in the induction of the lollipop monster phage as well as the mechanism by which canavanine depresses viral DNA synthesis. In this report, we demonstrate that the formation of lollipops involves a two step process: the induction step involving canavanine and a T4 late function, and the release or formation event for which arginine is required. The inhibition of DNA synthesis by canavanine appears to require protein synthesis.

MATERIALS AND METHODS

Growth conditions. Bacteriophages T4B and T4D

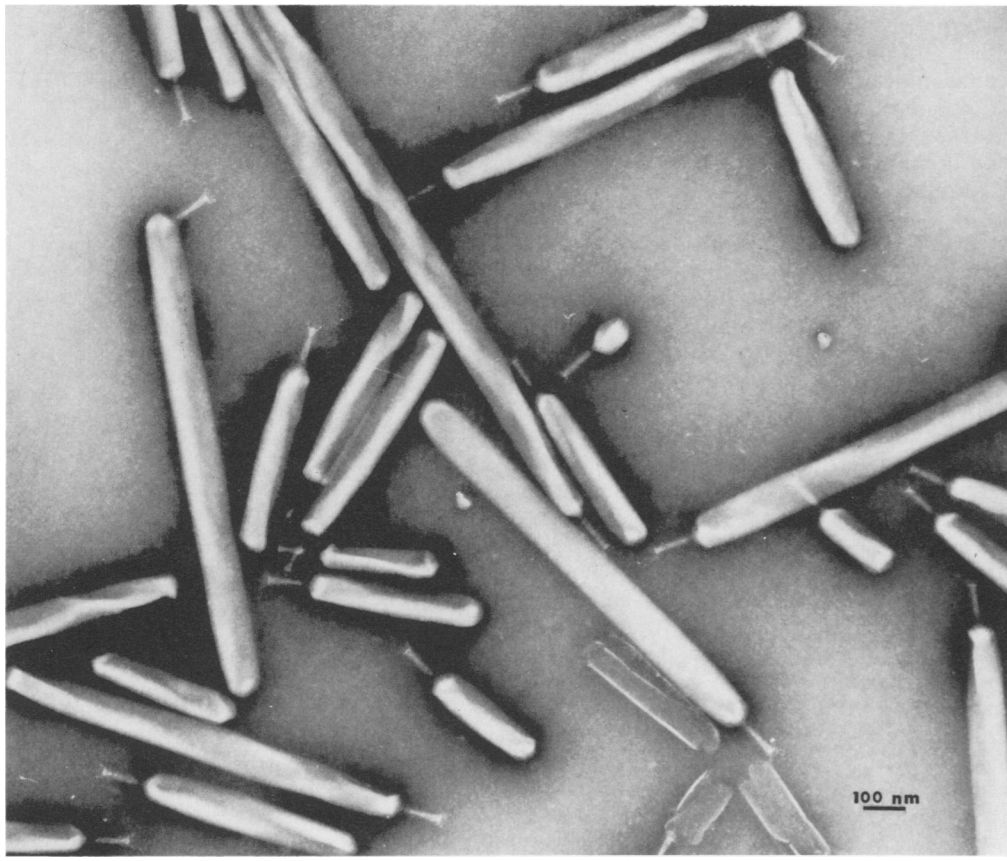

FIG. 1. *Electron micrograph of purified T4B lollipops.*

were grown at 37 C with aeration in minimal medium supplemented with amino acids (6). Unless noted, the host *Escherichia coli* B was infected with a multiplicity of infection of 5 phage/bacterium and then superinfected with the same amount of virus 7 to 9 min later. L-Canavanine sulfate (Calbiochem) and L-arginine (which will be referred to as canavanine and arginine) were added as noted in the text.

Measurement of DNA synthesis. In continuous labeling experiments, the cells were grown in minimal medium to 2×10^8 cells/ml. Prior to infection thymine was added (2 μg/ml final concentration) and 3 min after infection [^{3}H]thymine (10.3 Ci/mmol, New England Nuclear Corp.) was added to a final activity of 0.5 μCi/ml. In the canavanine dose response experiment, DNA synthesis was measured by adding [^{3}H]thymine to the samples at 35 min after infection for 5 min. In each case, the samples were precipitated with an equal volume of 10% trichloroacetic acid and after 30 min at 0 C were filtered on Whatman GF/A glass fiber filters and washed with 5% trichloroacetic acid. The filters were dried in glass vials, and 10 ml of toluene scintillation fluid (16.0 g of 2,5-diphenyloxazolyl, 0.2 g of 1,4-bis-(5-phenyloxazolyl)-benzene, 1 gallon of toluene) was added to each vial. Samples were counted on a Nuclear Chicago Mark I scintillation counter.

Other methods. Electron microscopy, using phosphotungstic acid negative staining (4), was performed as described previously (6). Lollipops were counted and measured from projections of negatives and the lengths were expressed in terms of normal phage head lengths obtained from the identical field. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by using a modification (30) of the procedures for thin slabs (29). The proteins were labeled by using [^{14}C]leucine (>270 mCi/mmol, New England Nuclear Corp., 0.67 μCi/ml final activity) as described in Fig. 3. The gel was dried and Kodak Brand Blue film was exposed for 10 days.

RESULTS

Effect of L-canavanine on the single burst. It has been shown that canavanine produces a 70 to 80% inhibition of T4 DNA synthesis (7) and reduces the phage yield 100- to 150-fold (9). When the effect of canavanine is relieved by the addition of arginine, the phage yield is restored to about 25% of the control culture and about 2.5% (T4B) of the phage population consists of lollipops. These lollipops contain about 17% of the assembled phage proteins. A series of single

burst experiments was performed to determine whether all infected cells were affected equally by canavanine or whether only a limited portion of the population was involved. If the latter case were true, the range of the single burst sizes should be similar to the untreated control cells. The number of single bursts in any one size interval would be markedly decreased. On the other hand, if canavanine affected the entire population of infected cells, the distribution of single burst sizes should be shifted such that the average single burst is decreased.

The modified single burst experiments were performed by using control infected cells, infected cells exposed to canavanine and infected cells in which the canavanine was chased with arginine. The cells were infected with 5 T4D phage per bacterium followed by the same amount of phage at 9 min postinfection (PI). One third of the culture was retained as a control while the remainder received 130 μg of canavanine per ml at 10 min PI. One-half of the canavanine-treated portion was then chased with 260 μg of arginine per ml at 25 min PI. At 15 min PI the control culture was diluted and divided into portions such that each portion contained approximately one infected bacterium. The cultures which received canavanine and canavanine followed by arginine were treated in a similar manner at 25 and 35 min PI, respectively. In each case, the samples were diluted into medium supplemented with concentrations of canavanine and arginine which were identical with those in the original medium. The diluted cultures were incubated for an additional 2.5 h at which time $CHCl_3$ was added to insure lysis of the cells. The $CHCl_3$ was evaporated from the medium and then the lysates were assayed.

The results of the single burst experiments are shown in Table 1. The single burst sizes from the control infected cells covered a wide range yielding an average burst size of 74 phage per bacterium. In the control samples, plates which did not contain a single plaque probably did not receive an infected bacterium. Plates having from one to four plaques probably received unadsorbed phage, whereas plates which had more than four plaques probably received an infected bacterium which gave a phage burst. In the culture which was treated with canavanine, the single bursts were skewed towards the lower range of burst sizes. No bacterium yielded a burst size greater than 60, whereas the average single burst size was only 8. In canavanine-treated cultures which were chased with arginine, the average single burst almost doubled to 15 phage/cell. The distribution of burst sizes shifted such that the average burst size was increased, indicating that the majority of the infected cell population was participating in the recovery upon addition of arginine.

TABLE 1. *Effect of canavanine on single burst size of T4-infected E. coli B*

Burst size	No. of bursts per interval		
	Control	Canavanine (no arginine)	Canavanine (arginine chase)
0	28	25	23
1-4	36	15	11
5-20	4	42	26
21-40	13	12	26
41-60	20	2	11
61-80	30	0	2
81-100	32	0	0
101-120	20	0	0
121-140	17	0	1
141-160	14	0	0
161-180	5	0	0
181-200	14	0	0
201-220	4	0	0
221-240	8	0	0
241-260	6	0	0
261-280	2	0	0
281-300	5	0	0
301-320	2	0	0
321-340	0	0	0
341-360	3	0	0
361-380	1	0	0
381-400	0	0	0
>400	7	0	0
Percent samples with no burst	10	26	23
Avg burst	74 (590)[a]	8 (5.6)	15 (108)
Total samples	271	96	100

[a] The value in parenthesis is the average burst in an undiluted culture under conditions favoring lysis inhibition.

The results did indicate that a small portion of the infected population failed to give a phage burst upon exposure to canavanine. In the control, approximately 10% of the plates did not show a plaque. However, 26% of the samples which received canavanine and 23% of the samples which received canavanine and were chased with arginine also failed to give plaques. This indicates that about 15% of the infected bacteria failed to produce infectious phage after an exposure to canavanine. The lethal event appeared to be irreversible, as an arginine chase failed to significantly affect these bacteria.

Therefore, this portion of the population would not be expected to participate in the induction of lollipops. The major portion of the population was affected equally by canavanine and by the arginine chase. These results indicate that the entire phage-producing population of infected bacteria participates in the production of lollipops.

Previous results have shown that 130 μg of canavanine per ml reduces the phage yield to a level less than 1% of the control. However, in the single burst experiment where lysis inhibition (13, 25) was not maintained, the average burst of the canavanine-treated cells was only reduced to 10% of the controls. The reason for this apparent contradiction is that in conditions favoring lysis inhibition, the untreated cells gave much larger burst sizes but when canavanine was added, the average burst size was not increased, possibly because canavanine interfered with the establishment or maintenance of lysis inhibition (Table 1).

Multiplicity of infection and the induction of lollipops. The DNA of the T4 lollipop appears to exist as a single concatamer of T4 DNA (E. Uhlenhopp et al., J. Mol. Biol., submitted for publication). Other workers have shown that recombination (26) may be necessary in the formation of concatamers generated during the T4 replicative cycle (16, 17). It is reasonable that recombination would be enhanced if more than one infecting T4 genome was present and since lollipop maturation would likely require a supply of concatameric DNA, it is conceivable that the multiplicity of infection might influence the production of lollipops. To investigate this possibility, cells were infected at low multiplicities (Table 2) and at high multiplicities (Table 3). Canavanine and arginine were added at various times as noted. The data in Tables 2 and 3 show that lollipops were formed with the same efficiency at both low and high multiplicities of infection. A comparison of the data in both tables show that T4B produced a higher percentage of lollipops than T4D except when canavanine was added at 5 min PI. This indicates that perhaps the induction mechanism is less efficient during early stages of the infection.

TABLE 2. *Percent lollipops found at low multiplicity of infection (T4D)*

Min PI[a]		Multiplicity of infection			
Canavanine addition	Arginine addition	0.1	0.2	0.5	5.0
10	25	1.1	1.1	0.9	1.3

[a] Concentrations of canavanine and arginine were 130 μg/ml and 260 μg/ml, respectively. The phage were sampled at 150 min PI.

TABLE 3. *Percent lollipops found at high multiplicity of infection (T4B)*

Min PI[a]		Multiplicity of infection		
Canavanine addition	Arginine addition	3	8	12
5	45	1.2	0.9	1.1
10	25	2.4	1.9	2.4
15	40	2.0	1.7	2.5

[a] Concentrations of canavanine and arginine were 130 μg/ml and 260 μg/ml, respectively. The phage were sampled at 150 min PI.

Minimum exposure to canavanine necessary to induce lollipops. Canavanine acts in some manner to induce the formation of lollipops which appear after an arginine chase. The above results suggest that the efficiency of the lollipop induction mechanism is not the same at all times within the replication cycle. To test this possibility, experiments were done to determine the minimum exposure to canavanine necessary to induce lollipops as a function of the progression of the viral maturation process. Measurements of the lollipop head lengths were made to determine the relationship between head length and exposure to canavanine. Three series of experiments were done. Canavanine was added at 5, 10, and 15 min PI and then arginine was added after various intervals. As is shown in Table 4, when canavanine was added at 10 or 15 min PI, a 3-min pulse of canavanine was sufficient for the subsequent induction of lollipops. However, when canavanine was added at 5 min PI, a 20- to 25-min pulse was required to induce lollipops. The data show that the percentage of lollipops as well as the average lollipop head length increased with increased exposures to canavanine. When canavanine was added at 5 min PI, the percentage of lollipops always remained low. Although the head lengths of the lollipops induced by adding canavanine at 5 min PI were not measured, the heads were consistently shorter than those induced at later times. Again, these data suggest that at early times in the infection, the lollipops induction step is not fully committed.

Appearance of lollipops after a canavanine pulse. At 10 min PI, a 3-min exposure to canavanine is sufficient to induce lollipops. The following experiment was done to determine the

TABLE 4. *Characteristics of T4B lollipops formed after minimal exposures to canavanine*

Time PI (min)		Lollipops (%)[b]	Avg head length (phage eq.)
Canavanine[a] addition	Arginine[a] addition		
5	20	<0.1	
5	25	0.5	ND[c]
5	30	0.8	ND
10	12	<0.1	
10	13	0.8	4.3
10	15	1.6	5.9
10	20	2.2	6.9
15	17	<0.1	
15	18	0.8	4.5
15	20	1.1	6.4
15	40	2.3	7.1

[a] Concentration of canavanine and arginine were 130 μg/ml and 260 μg/ml, respectively. The phage were sampled at 150 min PI.

[b] Approximately 1,000 phage were counted in each sample.

[c] ND, Not determined.

length of time required to produce lollipops after the induction step. Canavanine was added at 10 min PI followed by arginine at 25 min PI and samples were withdrawn as shown in Table 5. The samples were concentrated and assayed, and the average head length of the lollipops as well as the percentage of lollipops was determined. The production of phage and lollipops was first noted at 50 min PI, 25 min after the addition of arginine. The percentage of lollipops remained approximately the same throughout the infection, regardless of the measured phage titer. This may indicate that the commitment to form a lollipop is a random event within the maturation machinery. It is of interest to note that the average head length of the lollipop increases with time after the arginine addition. This indicates that the smaller lollipops matured first; it is possible that lollipops with longer heads were incomplete when the infectious cycle was interrupted but no such structures were noted.

Effect of increasing concentrations of L-canavanine. As has been mentioned, canavanine affects several aspects of the growth of T-even phage. In the study of each of these induced defects, it would be desirable to be able to describe each one independently of the other. In an attempt to do this, the effect of increasing the concentration of canavanine was studied with respect to each parameter. Figure 2 illustrates the effect of increasing concentrations of canavanine on T4 DNA synthesis, lollipop formation, and the phage yield. In each case, canavanine was added at 10 min PI and to measure lollipop formation, 260 μg of arginine per ml was added at 25 min PI. The data indicate that in each case, the concentration of canavanine which resulted in the most dramatic response was between 12 and 20 μg/ml. It should be noted that the induction of lollipops exhibits an "all or nothing" response. However, on occasion, the use of low concentrations of canavanine has resulted in lower percentages of lollipops. Lollipops induced by using low concentrations of canavanine had heads shorter

TABLE 5. *Appearance of T4B lollipops after a canavanine pulse*[a]

Time (min)		Phage titer[b]	Lollipops (%)[c]	Avg head length (phage eq.)
PI	Post arginine chase			
24	−1	2.5×10^8	<0.1	
30	5	4.1×10^8	<0.1	
40	15	5.5×10^8	<0.1	
50	25	7.9×10^8	2.1	4.2
60	35	2.5×10^9	2.1	4.8
80	55	9.4×10^{10}	2.1	4.9
100	75	2.3×10^{11}	2.5	6.8
120	95	3.2×10^{11}	1.6	7.7
150	125	3.9×10^{11}	2.2	7.7
150	No. canavanine	1.0×10^{12}	<0.1	

[a] Canavanine (130 μg/ml) was added at 10 min and 260 μg of arginine per ml was added at 25 min. The culture was sampled as noted.

[b] Samples were concentrated for microscope examination prior to titering.

[c] Approximately 1,000 phage were counted in each sample.

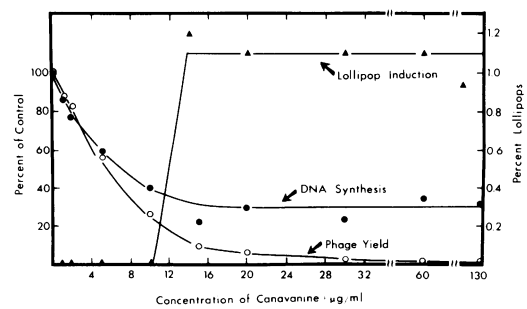

FIG. 2. *The effect of increasing concentration of canavanine on T4 DNA synthesis, phage yield, and the induction of lollipops. In each case, canavanine was added at 10 min PI. To measure lollipop induction, 260 μg of arginine per ml was added at 25 min PI. DNA synthesis was measured by [^{3}H]thymine incorporation for 5 min at 35 min PI.*

than those induced by using higher concentrations of canavanine. For these reasons, to induce lollipops, we routinely use from 130 to 200 μg of canavanine per ml and 260 to 400 μg of arginine per ml.

Figure 3 illustrates the effect of increasing concentrations of canavanine on the proteolytic cleavages involved in head assembly. This is dealt with more extensively in the companion paper. For the purpose of demonstrating the effect of increasing concentrations of canavanine on proteolytic cleavage reactions involved in head assembly, only the cleavage of the major head protein (P23) will be considered here. Canavanine at 5 μg/ml did not noticeably affect the cleavage of the major head protein. Most of the protein was present in the 45,000-dalton form (P23*). At 10 μg of canavanine per ml, there was a substantial increase in the 55,000-dalton form (P23) and at 20 μg of canavanine per ml most of the protein existed as the uncleaved P23 protein. The inhibition of proteolytic cleavage reactions, like the parameters described above, occurs in the presence of 10 to 20 μg of canavanine per ml. This concentration of canavanine is about 20% of the K_m value for canavanine transport by the arginine transport system in *E. coli* K12 (28).

L-Canavanine-induced inhibition of T4 DNA synthesis. The addition of 100 μg of canavanine per ml to a T4 infection inhibits DNA synthesis after a short lag (7). Our results have indicated that there is a required minimum exposure to canavanine in order to induce lollipops and that there is a lag of approximately 25 min after an arginine chase before lollipops and phage appear. These results suggest that the lollipop induction machinery must first incorporate canavanine into some unknown material and this results in the accumulation of a lollipop precursor substance(s). Before the lollipops can be matured the system must be released by some unknown series of events which requires approximately 25 min. The pos-

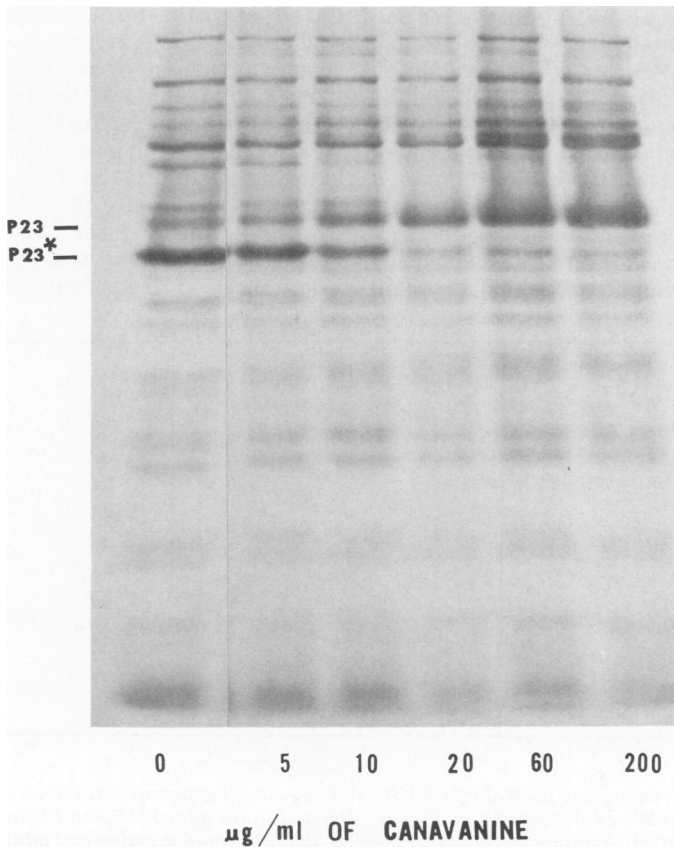

FIG. 3. *Autoradiogram of an SDS-10% polyacrylamide gel showing effect of increasing concentrations of canavanine on cleavage of the major head protein of T4 (P23). Canavanine was added at 10 min PI. [^{14}C]leucine was added at 15 min PI, and samples were isolated at 18 min PI.*

sibility that the restoration of DNA synthesis brought about by the addition of arginine could play a role in the release process led us to further investigate the canavanine-induced inhibition of T4 DNA synthesis. At various times after infection, 130 μg of canavanine per ml was added and was then subsequently chased by the addition of 260 μg of arginine per ml at several times after infection. Figure 4 shows that not only did canavanine result in a marked decrease in viral DNA synthesis but that the addition of arginine resulted in varying degrees of stimulation of DNA synthesis, depending on the time of rescue. When canavanine was added early in the infection, the stimulation which resulted after the addition of arginine was greater than when the canavanine was added late in infection. The addition of canavanine at 5 min PI followed by arginine at either 15 or 50 min PI resulted in a renewed rate of DNA synthesis comparable to the control rate. The net accumulation of viral DNA in the treated system eventually exceeded that of the control. The addition of canavanine at 10 min PI again illustrates the canavanine-induced inhibition of DNA synthesis (Fig. 4B). However, the addition of arginine failed to result in the dramatic stimulation of DNA synthesis illustrated in Fig. 4A. Less stimulation of DNA synthesis resulted if arginine was added at 30 min PI than if arginine was added at 16 min PI. Also, the net accumulation of DNA did not exceed the control as resulted when canavanine was added at 5 min PI. When canavanine was added at 15 min PI, the results were similar to those obtained when canavanine was added at 10 min PI except that the renewed rate of DNA synthesis and the accumulation of DNA after rescue was further reduced.

The results in Fig. 4 demonstrate that canavanine results in a reversible inhibition of DNA synthesis. In each instance, at least a 20-min lag was required after the addition of arginine before DNA synthesis was stimulated. Results shown in Fig. 4C demonstrate that the presence of arginine alone was not sufficient to restore DNA synthesis. When canavanine was added at 15 min PI, the inhibition of DNA synthesis occurred at approximately 30 min PI. The addition of arginine at 26 min PI did not preclude the inhibition. Clearly, the best interpretation of these results is that canavanine is incorporated into a compound required for DNA synthesis. After a lag of about 40 min the block was released, indicating that the inhibitory substance was diluted out or metabolized.

Does canavanine affect a protein required for DNA synthesis? The above results suggest that canavanine may have a specific effect on some compound necessary for DNA synthesis. A likely candidate would be a particular protein since it has been demonstrated that canavanine is incorporated into proteins (7, 10). To investigate this possibility, the effect of chloramphenicol on the inhibition of DNA synthesis mediated by canavanine and the stimulation of DNA synthesis by arginine was examined (Fig. 5). The addition of 50 μg of chloramphenicol per ml

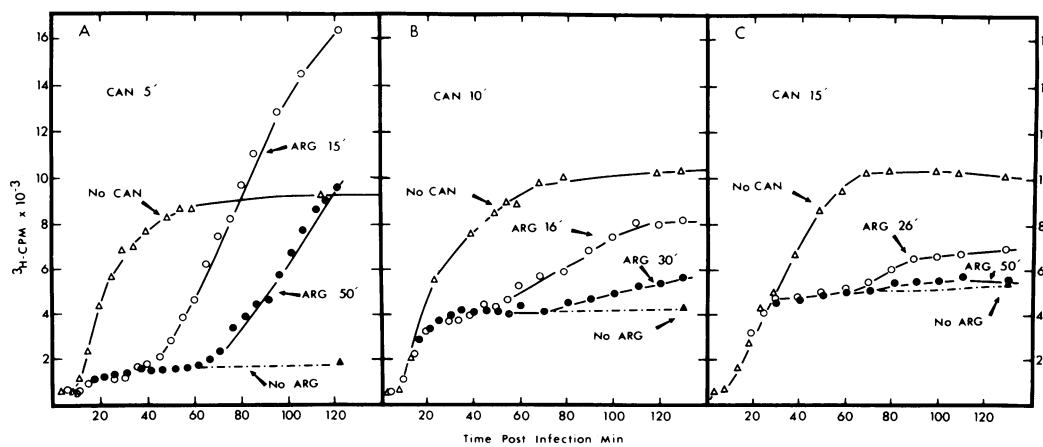

FIG. 4. *Effect of a canavanine pulse on T4 DNA synthesis. In all experiments, 130 μg of canavanine per ml was added, followed by 260 μg of arginine per ml. (A) Canavanine was added at 5 min PI and arginine was added at 15 min PI or 50 min PI. (B) Canavanine was added at 10 min PI and arginine was added at 16 min PI or 30 min PI. (C) Canavanine was added at 15 min PI and arginine was added at 26 min PI or 50 min PI. DNA synthesis was measured by continuous incorporation of [³H]thymine into acid-precipitable material. ARG, Arginine; CAN, canavanine.*

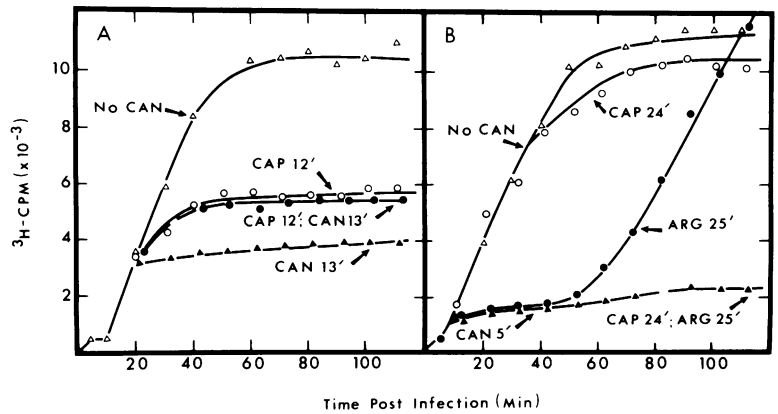

FIG. 5. *(A) Effect of chloramphenicol on the inhibition of T4 DNA synthesis by canavanine. Cells were infected at a density of 2 × 10^8 cells/ml. At 12 min PI, 50 μg of chloramphenicol per ml was added. At 13 min PI, 130 μg of canavanine per ml was added. (B) Effect of chloramphenicol on the stimulation of T4 DNA synthesis by arginine. Cells were infected at a density of 2 × 10^8 cells/ml. At 5 min PI, 130 μg of canavanine was added. At 24 min PI, 50 μg of chloramphenicol per ml was added and at 25 min PI, 260 μg of arginine per ml was added. ARG, Arginine; CAN, canavanine; CAP, chloramphenicol.*

at 12 min PI, just prior to the addition of canavanine at 13 min PI, prevented the inhibition of DNA synthesis mediated by canavanine (Fig. 5A). The addition of chloramphenicol resulted in an early cessation of DNA synthesis as compared to the control, but it is clear that canavanine alone decreased the rate of DNA synthesis much earlier than did chloramphenicol alone. In Fig. 5B it is clearly shown that the addition of chloramphenicol just prior to the addition of arginine prevented the stimulation of DNA synthesis.

The requirement for protein synthesis before arginine is able to stimulate DNA synthesis was further investigated (Fig. 6). Canavanine was added at 5 min PI followed by arginine at 25 min PI. Chloramphenicol was then added at various intervals after the addition of arginine. The data indicate that a 10-min period of protein synthesis was sufficient to allow some recovery of DNA synthesis. Although the DNA stimulation began at its characteristic time it was not able to sustain itself for more than a limited period. When chloramphenicol was added at 60 min PI, the rate of DNA synthesis paralleled the arginine-stimulated control rate for about 15 min. The rate then began to decrease rapidly, indicating a further requirement for protein synthesis to maintain the control rate. The DNA synthesis which is stimulated by the addition of arginine appeared to be more sensitive to chloramphenicol than is normal T4 DNA synthesis. In Fig. 5, chloramphenicol added at 25 min PI had a minor effect on T4 DNA synthesis. However, in cultures treated

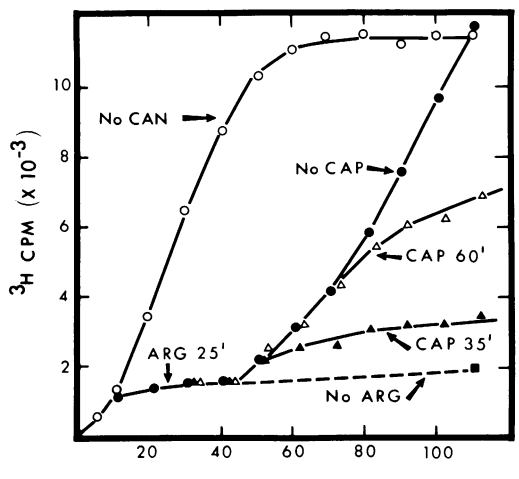

FIG. 6. *Effect of chloramphenicol on the resumption of T4D DNA synthesis after the addition of arginine. Cells were infected at a density of 2 × 10^8 cells/ml. At 5 min PI, 130 μg of canavanine per ml was added and 260 μg of arginine per ml was added at 25 min PI. At either 35 min PI or 60 min PI, 50 μg of chloramphenicol per ml was added. ARG, Arginine; CAN, canavanine; CAP, chloramphenicol.*

with canavanine, a 35-min period of protein synthesis allowed only about half of the DNA synthesis observed in the culture which did not receive chloramphenicol.

DISCUSSION

Our experiments describe parameters concerning the induction and appearance of the

canavanine-induced T4 monster phage, termed a lollipop. The data indicate that there is a two-step process involved in programming the formation of lollipops. The addition of canavanine constitutes the induction step. Although the induction step is not defined, canavanine results in the inhibition of cleavage reactions which occur during phage head assembly as well as a marked decrease in the rate of T4 DNA synthesis. There is a general disruption of the normal virus growth cycle. The release step is instigated by the addition of arginine. DNA synthesis is stimulated to varying degrees depending on when the canavanine is added and how long the infected cells are exposed to it. Phage and lollipops begin to appear approximately 25 min after the arginine chase when canavanine is added at 10 min PI.

The results that lollipops are produced at a constant percentage of the phage population throughout the maturation phase and that the majority of the infected cell population responds equally to the canavanine pulse suggest that the commitment to form a lollipop is a random event within the maturation process. However, the length of the lollipop head and the percentage of lollipops does vary with both the time at which canavanine is added to the infection as well as the length of exposure before the canavanine is chased with arginine. These results suggest that the probability of the commitment to form a lollipop is proportional to the amount of an undefined "precursor substance" which has been affected by canavanine.

The degree to which canavanine induces lollipops and inhibits DNA synthesis appears to be temporally controlled. The induction of lollipops reaches its greatest efficiency between 5 and 10 min PI. When canavanine is added at 5 min PI, a 20- to 25-min exposure is required to induce lollipops. However, at 10 min PI, only a 3-min pulse is required. The increased exposure to canavanine required for induction at 5 min PI indicates that (i) a relatively small amount of the necessary precursor(s) is present at that time and (ii) canavanine clearly prevents the step which is required to allow efficient induction of lollipops. Lollipops induced by adding canavanine at 5 min PI always have shorter heads than when canavanine is added at 10 to 15 min PI. This condition can be mimicked by pulsing with canavanine for short periods at 10 min PI, suggesting that the appearance of shorter lollipops is a reflection of lesser amounts of available "precursor substance." The arginine-reversibility of canavanine-inhibited DNA synthesis decreased between 5 and 10 min PI also. When canavanine is added at 5 min PI, DNA synthesis is inhibited to about the same degree as when canavanine is added at 10 to 15 min PI but the ability of the DNA synthesis to recover after the arginine chase is much greater at 5 min PI, even after long exposures to canavanine. Rapid T4 DNA synthesis begins at 7 to 8 min PI (25) and much of the temporal control of protein synthesis in the T4 infection is related to DNA synthesis (1, 5, 27). It may be that once T4 DNA synthesis has begun, it acquires an increased sensitivity to canavanine and that the active induction of lollipops requires a late protein precursor. When DNA synthesis is inhibited by canavanine, late proteins do accumulate (2, 7). The rescue event may be the release of the block on DNA synthesis which allows the lollipops and phage to mature.

Protein synthesis is required to release the canavanine-induced block of DNA synthesis and probably lollipop maturation as well. The finding that chloramphenicol prevents the inhibition mediated by canavanine and stimulation of DNA synthesis by arginine is intriguing. This finding suggests that canavanine is incorporated into a protein which is then inhibitory to DNA synthesis. However, in a normal infection, chloramphenicol does not inhibit T4 DNA synthesis at times when canavanine does inhibit. This suggests that new protein synthesis is not required to maintain DNA synthesis at these times which indicates that the proteins in the replication complex may be reusable but new proteins are constantly being added to the complex. The incorporation of a particular canavanyl protein could inactivate the complex and chloramphenicol could act by not allowing the faulty protein to be synthesized in one instance, or be replaced by functional protein in the chase with arginine.

The induction of lollipops clearly requires the accumulation of an undefined precursor substance(s). The lollipop precursor substance(s) could conceivably result from the accumulation of malfunctional proteins which have incorporated canavanine and have lost a control function. Alternatively, it could arise from the formation of an abnormally large head precursor structure assembled from normal proteins, which arises as the result of a specific malfunction of a specific length-controlling protein. The formation of lollipops upon the addition of arginine may require the synthesis of a necessary component which is lacking in the presence of canavanine. These possibilities are discussed in the companion paper (2).

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