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Function of T4D Structural Dihydrofolate Reductase in Bacteriophage Infection

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Various properties of the bacteriophage structural dihydrofolate reductase (DFR) have been examined to determine its function during phage infection. It has been found that a binding site for reduced nicotinamide adenine dinucleotide phosphate (NADPH), most likely on the DFR present in the phage tail plate, is required for phage viability. Attachment of adenosine diphosphoribose, an analogue of NADPH, to this site prevents phage adsorption and injection. This adenosine diphosphoribose inhibition can be competitively reversed by the addition of NADPH or oxidized nicotinamide adenine dinucleotide phosphate. It is suggested that, during phage infection, the host bacterial cell might leak compounds functionally similar to the pyridine nucleotides. These compounds have been shown to nonenzymatically change the conformation of the phage tail plate DFR which is apparently necessary for successful injection.

Kozloff et al. reported that bacteriophage T4D-induced dihydrofolate reductase (DFR) was an integral part of the phage particle and that its inclusion in the phage tail plate was essential for phage assembly and viability (6). Mathews confirmed the presence of phage DFR as a structural component by showing that the heat sensitivities of whole phage particles were due to the particular DFR gene they contained (7).

The presence of DFR in the phage tail plate, along with its substrate dihydropteroylhexaglutamate, is most unexpected. Immediately one can ask what role(s) phage DFR is playing as a structural component. Knowing that phage are inactivated by reduced nicotinamide adenine dinucleotide phosphate (NADPH; 6), a substrate for DFR, and that NADPH attachment can alter DFR conformation (1), Kozloff et al. proposed that the phage structural DFR could be involved in phage adsorption or injection, or both. It was also suggested that NADPH, presumably from cell leakage, bound to tail plate DFR, reducing the dihydropteroylhexaglutamate to the tetrahydro-compound and further that accompanying the reduction there was a conformational change of the enzyme which was reflected by a morphological alteration of the tail plate (6). In view of the observations of Greenfield et al. (2) that conformational changes of *Escherichia coli* DFR need not involve enzymic reduction, reduction to the tetrahydro-compound may not be required.

The above facts and conjectures lend themselves to two predictions. (i) If a NADPH-induced conformational change of DFR is essential for phage attachment or injection, or both, then the DFR of every potentially infectious particle must be able to react with NADPH. The ability of NADPH to bind to DFR can be determined by measuring the inactivation of whole phage particles. This NADPH inactivation is probably not physiologically significant, but it is a convenient method to measure the ability of phage DFR to bind NADPH. Thus, if binding of NADPH is essential for phage infectivity, every infective phage article must contain DFR which can bind NADPH, and one should not be able to isolate phage particles resistant to NADPH inactivation. (ii) A structural analogue of NADPH might bind to DFR reversibly, or irreversibly, and effect an alteration of phage adsorption or injection, or both. If an alteration was found and the analogue was reversibly bound to DFR, then added NADPH should compete with the analogue to restore normal phage infection.

Examination of the above predictions indicates that phage T4D structural DFR participates nonenzymatically in phage infection, probably by virtue of its ability to undergo a conformational change as a result of interaction with pyridine and adenine nucleotides.

MATERIALS AND METHODS

Bacteriophage and bacteria. These have been described in a previous paper (5).

Determination of adsorption and injection rates. Bacteria were grown overnight at 37 C in tryptone broth (Difco) plus 0.5% NaCl, Fresh logphase cells (2×10^8 to 4×10^8/ml) were sedimented once and suspended in tryptone broth with the aid of a Vortex mixer. Most experiments were done at room temperature (20–22 C), since this slowed the infection process and facilitated accurate measurements. Phage were added to bacteria at a multiplicity of infection of 0.1. The total volume of the contents of the adsorption tube ranged from 0.2 to 0.5 ml in various experiments. Within a single experiment, the amount of bacteria and the total volume were kept constant. At designated times, 0.02-ml samples were removed to tubes in an ice bath which contained 2.0 ml of tryptone broth without NaCl (SFB; salt-free broth). One-milliliter samples were blended in the microattachment container of a Sorvall Omnimixer. Cell viability is unaffected by the blending treatment. The containers were prechilled on ice and were immersed in an ice bath during blending. Cell-phage mixtures were blended for 1 min at a setting of 5 (9,000 rpm), and 0.05 ml of the blended mixture was placed in a tube containing 0.45 ml of T4D antiserum. A separate tube containing 0.45 ml of antiserum received 0.05 ml of the ice-cold unblended adsorption mixture. T4D antisera with different K values were used throughout the course of these experiments. Preliminary experiments established the dilution of antisera (in SFB) which would inactivate about 5×10^4 T4D particles per ml within 5 min at room temperature. Antisera potency was checked periodically. Blended and unblended samples were incubated in antiserum for 10 min, diluted 10-fold into SFB and plated in the usual manner. Adsorption rates were obtained from the plaque counts (infected cells) of unblended samples. The fraction of adsorbed phage which had injected their DNA was calculated for a given sample by dividing the plaque count of the unblended sample by the plaque count of the blended sample.

The pH of unadjusted tryptone broth was slightly below neutrality (pH 6.8–6.9) and is referred to as pH 7 in this report. When adsorption and injection rates were done at pH 5, tryptone broth adjusted to pH 5 with HCl was used for resuspension of the log phase cells used in the adsorption tube.

Competitive inhibition of adsorption and injection. Solutions of adenosine diphosphoribose (ADPR) and other compounds were prepared at a concentration of 10 times the value desired in the adsorption tube and diluted 10-fold into the adsorption tube mixture (i.e., 0.025 ml of 0.05 M ADPR into a final volume of 0.25 ml to give a final ADPR concentration of 5 mM). The pH of all solutions was determined, and the pH was adjusted with HCl or NaOH when necessary. All compounds being tested in a particular adsorption mixture were added to cells before addition of phage.

Inactivation of T4D particles. Inactivation of T4D at pH 5 with NADPH was done by adding 0.03 ml of 0.03 M NADPH in 0.5 M ammonium acetate buffer (pH 5) to 0.27 ml of a phage suspension which had been dialyzed for 18 h against 0.05 M ammonium acetate buffer (pH 5). The incubation mixture was overlaid with mineral oil to prevent air oxidation of the NADPH.

Heat inactivation of T4D in the presence of ADPR or NADPH was done in 0.1 M potassium phosphate buffer (pH 7.0) at 60 C. Phage were diluted so that the initial phage concentration in the reaction mixture was about 10^7 particles per milliliter.

Other methods (chemicals). The hydroxylamine mutagenesis method of Hall and Tessman was used (4). Isolation of soluble DFR and determination of enzymatic activity by a spectrophotofluorometric method were as described earlier (6). All pyridine nucleotides and ADPR were products of P. L. Biochemicals. Adenosine diphosphoglucose (ADPG), adenosine diphosphomannose (ADPM), and nicotinamide mononucleotide (NMN) were purchased from Sigma Chemical Co.

RESULTS

Attempts to isolate a T4D NADPH resistant mutant. Large numbers of phage were treated with NADPH at pH 5. In the absence of NADPH the phage are stable for at least 24 h. Phage were inactivated with NADPH at pH 5 rather than at pH 7 because at pH 5 inactivation was faster and exponential (no initial lag, reference 6). All of the particles were inactivated exponentially within 24 h, and 10^{12} particles were completely inactivated in other experiments (Fig. 1). One can conclude that if a mutation to NADPH resistance can occur, (i) the frequency of such a mutation is less than 10^{-12} or, (ii) such a mutation is lethal.

One hundred T4D DFR (wh) mutants have been isolated following hydroxylamine mutagenesis. The mutants were classified as DFR mutants according to their distinctive plaque morphology (3). It was felt that the frequency of NADPH resistant mutants might be enriched in this collection of 100 DFR mutants. However, when the inactivation of each mutant by 3 mM NADPH in phosphate buffer (pH 7.2, 37 C) was examined, no resistant mutants were found.

The sensitivity of T4D particles to NADPH plus the lack of NADPH resistant mutants indicated that the ability of phage DFR to react with NADPH was essential for viability.

The effect of ADPR on phage adsorption and injection rates. The first structural analogue chosen for study was ADPR. ADPR lacks the nicotinamide portion of the pyridine nucleotide structure. At a concentration of 5 mM, ADPR inhibited phage adsorption, but at 1 mM ADPR there was no effect on the rates of phage adsorption or DNA injection. Intermediate concentrations of ADPR inhibited both adsorption and injection rates (Table 1). Whereas ADPR decreases the rate of adsorption at 1 min (2.5 mM ADPR, Table 1; 3 mM ADPR in other experiments), the amount of adsorption is equal to that of the control (no ADPR) at 6 min. Typically the injection process

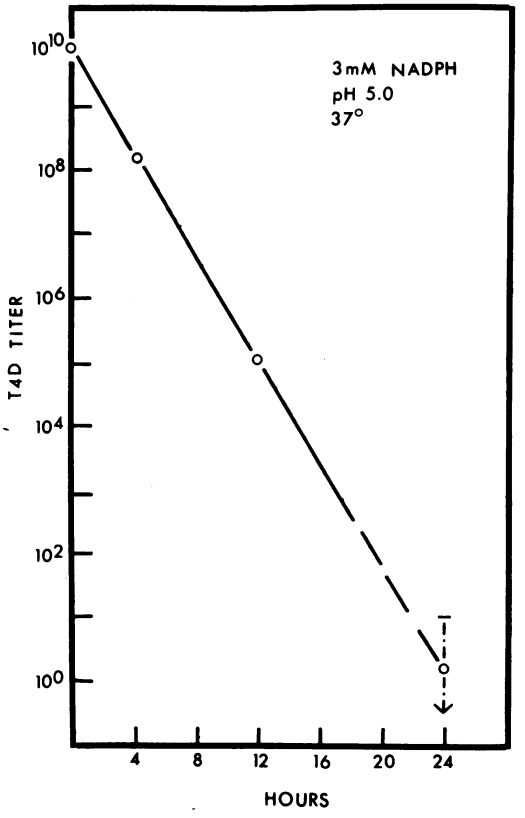

FIG. 1. *Inactivation of T4D by NADPH. Inactivation by 3 mM NADPH was carried out in 0.05 M acetate buffer (pH 5) under oil.*

TABLE 1. *Effect of ADPR on adsorption and injection rates*[a]

Experiment	ADPR concn (mM)	Adsorption (%)		Injection (%)	
		1 min	6 min	1 min	6 min
1	0	43	91	45	77
	1	55	97	41	92
	2.5	20	88	33	56
	5.0	0	0	0	0
2	0	33	66	45	81
	2.5	20	74	35	67
	5.0	0	0	0	0

[a] Adsorption based on phage input of 4.65×10^7 per ml. Percent injection = (infected cells, blended/infected cells, unblended) 100.

was still impaired at 6 min relative to control values.

In subsequent experiments ADPR was used at 5 mM because this concentration was the minimal amount needed to completely inhibit phage adsorption in 90% of the experiments, and one could be assured that there was no large excess of ADPR in the system. In 10% of the experiments, 5 mM ADPR did not give complete inhibition, and a few plaques appeared (about 1–3% of control values at any time point). This variability was of some importance only with respect to data presented in Table 2 and will be discussed in that section.

In vitro enzyme assays strongly suggested that the ADPR effect on adsorption and injection was related to the specific binding of ADPR to phage structural DFR. Phage-induced DFR was partially purified, and the ability of ADPR to bind and inhibit enzymatic activity of the soluble enzyme was tested. The results in Fig. 2 confirm that ADPR does bind and inhibit enzymatic activity. Comparison of Fig. 2 with Table 1 shows a good correlation between the concentration of ADPR needed to inhibit enzyme activity (5 mM inhibited 95%) and the concentration of ADPR needed to inhibit the adsorption of whole phage particles containing DFR (5 mM inhibited adsorption about 98%).

Assured that ADPR was binding to DFR, probably at the NADPH binding site, NADPH was added with ADPR to cell-phage mixtures to determine whether NADPH could compete with ADPR for the binding site. At NADPH concentrations from 1 to 10 mM and at pH 7, phage are not inactivated for several hours. The results of

TABLE 2. *Ability of various compounds to relieve 5 mM ADPR inhibition of T4D adsorption*

Compound	Concn (mM)	Relative ability[a]
Group I		
NAD^+	5	Poor
NADH	5	Poor
$NADP^+$	5	Excellent
NADPH	5	Excellent
Group II		
Adenine	5	Moderate
Adenosine	5	Poor
AMP	5	Excellent
ADP	5	Poor
ATP	5	Excellent
Group III		
Pyridine	20	Variable
Nicotinic acid	20	Variable
Nicotinamide	20	Variable
Iso-nicotinamide	20	Poor
$N\text{-}CH_3$ nicotinamide	20	Poor
$I\text{-}CH_3$ nicotinamide	20	Poor
Pyridine-3-sulfonic acid	20	Poor

[a] The terms poor, moderate, excellent, and variable are defined in the text.

a typical experiment are shown in Fig. 3 and 4. These experiments were carried out at 18 C, although identical results were obtained at 22 C. NADPH (3 mM) restores adsorption only slightly at 6 min, whereas 10 mM NADPH not only completely relieves the ADPR inhibition but, in fact, stimulates the adsorption rate in a manner which is not presently understood (Fig. 3). Although it is not shown in this experiment, addition of 5 mM NADPH restored adsorption rates to those of the control (no additions). Addition of 3 mM NADPH was effective in restoring the injection of phage DNA. At 6 min, the amount of injection was about 80% of the control value (Fig. 4). Addition of 5 mM NADPH (not shown) or 10 mM NADPH fully restored injection rates to those seen with the control. The results shown in Figures 2, 3, and 4 strongly suggest that ADPR is a competitive inhibitor of NADPH and that binding of NADPH to the phage particle may be essential for phage infection to occur.

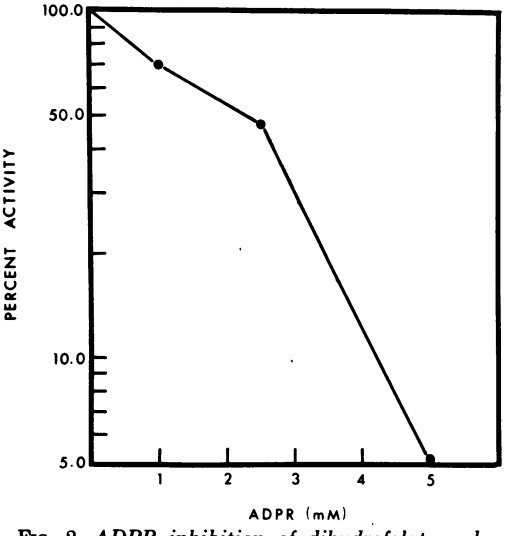

FIG. 2. *ADPR inhibition of dihydrofolate reductase activity. The spectrophotofluorometric method used has been described (6).*

Ability of various compounds to inhibit phage adsorption or relieve ADPR inhibition of phage adsorption. To gain some insight into the binding specificities, the ability of a variety of compounds structurally related to NADPH to either inhibit phage adsorption or relieve ADPR inhibition of adsorption was studied. The results are shown in Table 2. None of the compounds listed in Table 2 inhibited phage adsorption. The terms poor, moderate, and excellent refer to a compound's ability to relieve ADPR inhibition relative to a control adsorption (no additions) done with the same cells on the same day. The term poor indicates that the compound relieved the ADPR inhibition of adsorption to about 10% of the level of the control; the term moderate indicates that the compound relieved ADPR inhibition of adsorption to about 50% of the control; and the term excellent indicates that the compound completely relieved the 5 mM ADPR inhibition of phage adsorption (i.e., was equal to the control; no additions).

In the first group of compounds, the pyridine nucleotides, it was significant that oxidized

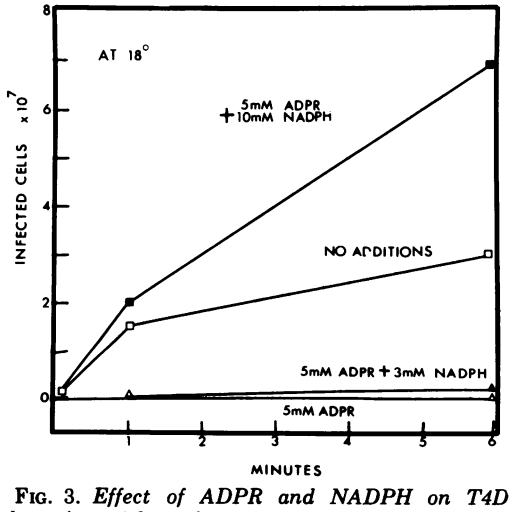

FIG. 3. *Effect of ADPR and NADPH on T4D adsorption. Adsorption was carried out in pH 7 tryptone broth at 18 C.*

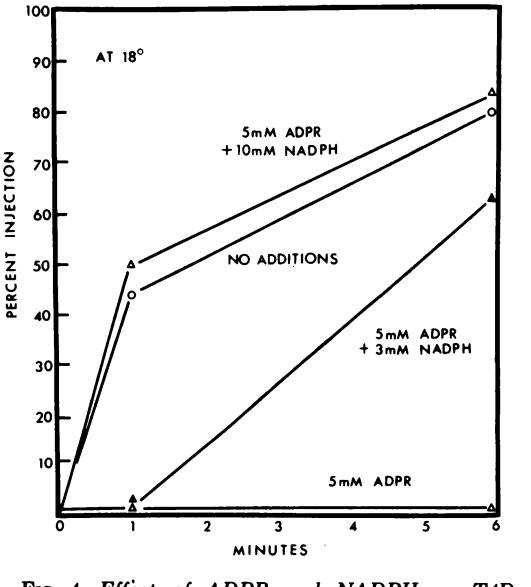

FIG. 4. *Effect of ADPR and NADPH on T4D injection. The experiment was done in pH 7 tryptone broth at 18 C.*

nicotinamide adenine dinucleotide phosphate ($NADP^+$) was as effective as NADPH on an equimillimolar basis in competing with ADPR to restore adsorption. Although it is not shown in Table 2, $NADP^+$ also restored injection rates to those obtained with a control culture. The ability of $NADP^+$ to effectively restore both adsorption and injection clearly indicates that phage structural DFR is functioning nonenzymatically in both processes since the oxidized form, $NADP^+$, cannot reduce dihydropteroylhexaglutamate to tetrahydropteroylhexaglutamate. The observation of Greenfield et al. that conformational changes of DFR can occur without enzymatic reduction of dihydrofolate supports this conclusion (2).

Of the adenine-related compounds in Group II, AMP and ATP effectively competed with 5 mM ADPR to restore phage adsorption.

The third group of compounds, analogues of pyridine, were ineffective in competing with ADPR. When ADPR inhibition was complete, as determined in a control experiment, pyridine, nicotinamide, and nicotinic acid were poor competitors. When ADPR inhibition was incomplete, their abilities to compete with ADPR ranged from moderate to excellent. These variable compounds were less effective than NADPH, $NADP^+$, AMP, and ATP in competing with ADPR since Group III compounds were tested at a concentration of 20 mM. This variable response was not seen with the other four compounds in Group III or with any other compounds in Table 2.

Also tested were the ADPR related compounds, ADPM and ADPG. Neither compound inhibited phage adsorption when tested at a concentration of 5 mM, whereas 5 mM ADPR completely inhibited phage attachment. Also, ADPM and ADPG did not bind to phage DFR in vitro as evidenced by unimpaired enzymatic activity of the DFR. The fact that neither ADPG nor ADPM inhibit phage adsorption or DFR enzymatic activity demonstrates the specificity of the terminal ribose portion of ADPR as being a crucial determinent in binding to DFR.

NMN (nicotinamide with an attached ribose and phosphate) was the only other compound tested besides ADPR which inhibited phage adsorption. Initial experiments show that, like ADPR, NMN inhibits enzymatic activity of DFR in vitro. Since addition of nicotinamide to a phage-cell mixture does not inhibit adsorption and NMN does, the importance of the ribose portion of NMN (structurally the terminal ribose of ADPR) is again demonstrated.

These effects of NMN added to those of ADPR clearly support the conclusion that a binding site exists on the phage structure for compounds containing the components nicotinamide-ribose-P-P-ribose-adenine. A compound containing a specific ribose (ribose attached to NMN, terminal ribose of ADPR) but not the rest of the pyridine nucleotide structure inhibits phage adsorption and injection. Competition of these inhibitors by the complete pyridine coenzymes, NADPH and $NADP^+$, and other analogues structurally related to NADPH and NADP but not containing the critical ribose moiety support the above conclusion regarding a pyridine nucleotide binding site. DFR is the most likely and probable component of the tail plate to have such a specific site.

Effect of pH 5 broth on phage adsorption and injection. Since it is known: (i) that isolated phage tail plates are sensitive to acid (C. J. Male, V. A. Chapman, S. S. DeLong, and L. M. Kozloff, Bacteriol. Proc. p. 183, 1970); (ii) that the rate of phage inactivation by NADPH is four times faster at pH 5 than at pH 7 (6); and (iii) that the rate of phage inactivation by antisera to phage DFR is twice as fast at pH 5 as at pH 7.2 (unpublished experiments), it seemed reasonable to assume that the structure of the phage tail plate is altered at pH 5. Incubation at pH 5, either by structural alteration of proteins in the tail plate or by a direct conformational change of DFR, changes the reactivity of the phage DFR with respect to substrate binding and antisera inactivation. It thus seemed plausible that at pH 5 phage infection might be inhibited. Comparison of lines 1 and 2 of Table 3 shows that both adsorption and injection are inhibited at 1 min at pH 5. The amounts at 6 min are close to those of the pH 7 control sample. The pH 5-mediated decrease of adsorption and injection rates can be abolished by the addition of 3 mM NADPH (line 3). Addition of 1 mM ADPR to a phage-cell suspension at

TABLE 3. *Effect of incubation at pH 5 on adsorption and injection*[a]

Experimental conditions	Adsorption (%)		Injection (%)	
	1 min	6 min	1 min	6 min
pH 7	77	100	39	71
pH 5	26	65	27	69
pH 5 + 3 mM NADPH	60	86	49	83
pH 5 + 1 mM ADPR	0	4	0	47
pH 5 + 1 mM ADPR + 3 mM NADPH	46	96	45	64

[a] Adsorption based on phage input of 4.65×10^7 per ml. Percent injection = (infected cells, blended/infected cells, unblended) 100.

pH 5 completely inhibited phage attachment (line 4). One mM ADPR did not affect adsorption or injection at pH 7 (Table 1). The combined inhibitions due to pH 5 broth and 1 mM ADPR are relieved by 3 mM NADPH as shown in line 5 of Table 3. Presumably, binding of NADPH to DFR "protects" the DFR or associated proteins from the conformational change induced by treatment of pH 5.

Effect of NADPH and ADPR on heat inactivation of T4D phage particles. The evidence presented above that a conformational change of DFR is associated with phage infection is circumstantial but attractive. It is difficult to demonstrate the conformational change of a structural protein in situ, but heat inactivation experiments below offer independent evidence that binding of either ADPR or NADPH to structural DFR is accompanied by a conformational change.

It is well known that the heat sensitivity of a protein is related to its tertiary structure. With respect to structural DFR, Kozloff et al. first demonstrated a correlation between the heat sensitivity of a whole phage particle and the structural DFR it contained (6). This discovery was expanded and confirmed by the genetic and biochemical studies of Mathews (7). Thus one might expect to find differences in the heat sensitivity of particles treated with ADPR or NADPH if their binding to DFR induced a conformational change. Phage particles in the presence of 3 mM NADPH are more sensitive to heat inactivation than in the absence of NADPH (Fig. 5). Similarly, phage particles heated in the presence of 3 mM ADPR were 100-fold more sensitive than phage heated in the absence of ADPR (measured at 30 min).

Ability of ADPR to inhibit phage adsorption as a function of time of addition. ADPR (2.5 mM) decreased the rates of both phage adsorption and injection (Table 1). But upon continued incubation, adsorption and injection rates approached those seen in the absence of ADPR. Restoration of these rates to normal, as a function of time, could reflect the increasing concentration of a compound(s) which competes with ADPR for the NADPH binding site on phage DFR. Thus, one could predict that the sensitivity of a phage-cell suspension to ADPR inhibition of adsorption might decrease with time. The experiment shown in Fig. 6 supports this hypothesis. In this experiment cells and phage were mixed, and either ADPR or broth was added at 15 or 45 s. Adsorption was identical in the two broth controls. If ADPR was added at 15 s, there was an immediate inhibition of phage attachment, whereas if ADPR was added at 45 s the phage were much more resistant to ADPR inhibition. Identical results were obtained in two separate experiments. Phage attachment in the absence of ADPR was linear through 1 min, which makes the difference in ADPR inhibition valid. The data in Fig. 6 suggests that there is an increased concentration of a compound(s) present between 45 and 60 s as compared to between 15 and 30 s, which competes with ADPR for the DFR binding site. After these 15-s intervals, the adsorption rates are approximately the same and show the inhibiting action of the ADPR.

Of the compounds tested, NADPH, $NADP^+$, AMP, and ATP, all normal bacterial cell constituents, were effective in competing with ADPR. Thus it appears that the physiologically active compound(s) needed to bind to phage DFR and induce the conformational change necessary for successful phage infection might be a product of cell leakage.

DISCUSSION

Several lines of evidence indicate that the NADPH and ADPR binding site of the phage particle is the phage structural DFR. (i) Phage-induced DFR is a structural component of the phage particle (6, 7), and no other protein (enzyme) with a NADPH binding site is known to be a phage structural component. (ii) ADPR and NMN inhibit phage infection, and both compounds inhibit enzymatic activity of soluble DFR in vitro. (iii) Heat sensitivity of phage particles is correlated with the product of the DFR gene (7), and both ADPR and NADPH

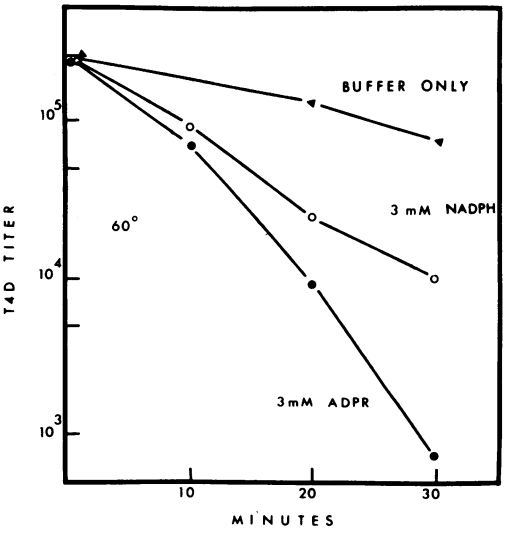

FIG. 5. *Effect of ADPR and NADPH on heat inactivation of T4D. Inactivation was carried out in 0.1 M phosphate buffer (pH 7) at 60 C.*

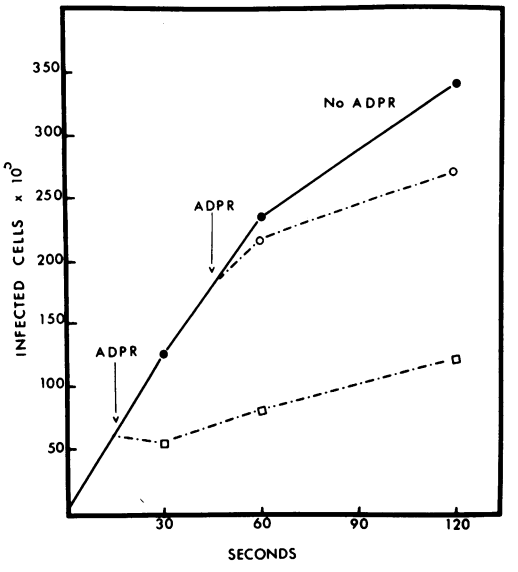

FIG. 6. *Effect of time of addition of ADPR on T4D adsorption. Four adsorption tubes were prepared (phage at a multiplicity of infection of 0.1). ADPR (5 mM) was added to one tube at 15 s after mixing cells and phage, and 0.02-ml samples were removed at 30, 60, and 120 s to 2 ml of cold SFB and processed as described in Materials and Methods. ADPR (5 mM) was added to a second tube at 45 s, and samples were removed at 60 and 120 s. The results of the control (broth added at 15 s or 45 s) are shown as one curve since the separate control curves were superimposable.*

treatment alter heat sensitivity by presumably inducing a conformational change of the structural DFR.

ADPR appears to be useful as a probe to investigate the role of the phage tail plate structural DFR in infection. By virtue of its binding to DFR, it blocks infection and creates an artificial requirement for molecules which both can displace ADPR by competition and which can function physiologically in allowing infection to proceed normally. Clearly, ADPR inhibits both adsorption and injection, and the inhibition of both processes can be relieved by the addition of various compounds.

The data suggests that ADPR acts differently with respect to inhibition of adsorption and injection. It was shown (Table 1) that after 6 min of incubation with ADPR, the amount of adsorption was equal to that reached in the absence of ADPR, whereas the amount of injection still remained low. Thus injection is more sensitive to the ADPR-induced conformational change of DFR than is adsorption. In addition, 3 mM NADPH (Fig. 3 and 4) was virtually ineffective in restoring the amount of adsorption after 6 min, whereas the same concentration of NADPH restored injection to 80% of the normal value. Thus the injection process is much more sensitive to the NADPH-induced conformational change of DFR than is the adsorption process, which is what would be predicted if indeed a NADPH ($NADP^+$, etc.)-induced conformational change was obligatory for injection but not for adsorption.

It is quite possible that the ADPR-induced conformational change of DFR blocks adsorption by not allowing for proper orientation of tail fibers. Since all of the input phage were recovered when the reaction was not stopped by dilution and antisera treatment, it is clear that ADPR does not cause an abortive adsorption, but rather that adsorption does not occur. Thus, we interpret the effectiveness of NADPH, NAD^+, etc., to indicate not that these molecules are required for adsorption but rather that by displacing ADPR they allow DFR to participate normally in tail fiber orientation. This "normal" participation of DFR in fiber orientation does not require a NADPH ($NADP^+$, etc.)-induced conformational change. It occurs as a result of the plasticity of the DFR molecule.

Infection of a bacterium by phage T4D involves numerous complex steps (11, 12). The precise sequence of events is not clearly established, but it is apparent that an exact temporal sequence of events exists to ensure successful phage infection. It is also generally accepted that phage infection alters host membrane permeability and that leakage of cell contents occurs (8, 9. 10). Recent evidence of Weintraub and Frankel showed that phage-induced proteins became associated with the host membrane in as little as 4 min after infection. It was implied that these new phage proteins may be involved in sealing the membrane and restoring its permeability functions (13). No systematic studies on the appearance and nature of early leakage products has been done.

The next question to be dealt with is the identity of cell leakage product(s) which might participate in the injection process. Kozloff et al. proposed that NADPH might be the physiologically active cell leakage product. Since it was shown here that the oxidized form, $NADP^+$, is as an effective ADPR competitor as NADPH, enzymatic activity of DFR is not essential for adsorption or injection. It has been found that adsorption and injection of phage T4Dwhl, a DFR mutant which infects "normally" and has enzymatically inactive DFR in its base plate (6), is also inhibited by ADPR and the inhibition is relieved by NADPH.

Of the compounds tested (Table 2) for their

ability of relieve ADPR inhibition of T4D adsorption, NADPH, $NADP^+$, AMP, and ATP were most effective. All of these compounds are present in *E. coli*, and Sekiguchi (10) and Puck and Lee (8, 9) showed that the pyridine and adenine nucleotides are cell-leakage products resulting from phage infection. These cell-leakage products presumably can all react with the phage DFR and initiate the tail plate conformational change which leads to successful infection.

ACKNOWLEDGMENTS

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