# THERMODYNAMIC AND KINETIC STUDIES ON THE ATTACHMENT OF T1 BACTERIOPHAGE TO BACTERIA*

by

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The first phase of the invasion of *E. coli* strain B by T1 bacteriophage has been found to proceed in two distinct, consecutive steps[1,2], of which the first is a reversible binding of the phage to the bacterium through the interaction primarily of ionic groups on the two surfaces[3,4]. Under optimal conditions at 37° C this reaction has a collision efficiency in the neighbourhood of 100% (*i.e.*, almost every random contact between the reactants results in attachment). This high efficiency remains unchanged as the temperature is lowered from 37 to 2° C, showing that no significant activation energy is required. Reversible attachment is followed by an irreversible step, after which the phage can no longer be recovered as an infective particle. This step, in contrast to the preceding one, requires around 18,000 calories/mole of activation energy, and yet also occurs with an almost 100% collision efficiency at 37° C. As will be shown in the present work, this result is a consequence of the stepwise nature of the reaction.

These two steps of attachment may be represented as follows:

$$V + C \underset{k_2}{\overset{k_1}{\rightleftarrows}} VC \qquad (1)$$

$$VC \xrightarrow{k_3} X \qquad (2)$$

where $V$ represents T1 virus; $C$, the host cells; $VC$, reversibly-attached virus, and $X$, the irreversible virus-cell complex. The symbols, $k_1$, $k_2$, and $k_3$ refer to the specific rate constants for the reactions indicated. These equations apply under conditions where the virus/cell ratio is sufficiently low so that no cell binds more than one virus particle.

Four methods have been found[2] which make it possible selectively to inhibit the second, irreversible reaction without impairing the efficiency of the first step: a. lowering of the reaction temperature to 0° C; b. irradiation of the bacteria with a high dose of ultraviolet light; c. previous treatment of the bacteria with $Zn^{+2}$ ions; and d. substitution of the bacterial mutant, *E. coli* strain B/1 (tryptophan-deficient), for the normal wild-type strain B. All of these measures specifically block the irreversible step[2] and so permit

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isolation of the initial reaction. In most of the experiments here presented, the second step was blocked through use of the mutant B/1. Under these conditions it will be shown the first step attains a state of thermodynamic equilibrium. From measurements of the equilibrium constant as a function of temperature, values for the change in standard free-energy, heat of reaction, and entropy have been calculated. In addition, values for the rate constants, $k_1$ and $k_2$ in equation (1), are reported.

## MATERIALS AND METHODS

T1 bacteriophage, *E. coli* strain B bacteria, and a mutant strain B/1 (tryptophan-deficient) derived from B (which is resistant to infection by T1 but sensitive to all other members of the T-series), have been used in these investigations. Details of the procedures involved in their preparation have been described in detail[5]. The bacteria were grown in nutrient broth (Difco) + added 0.5% NaCl for 18 hours at 37° C with aeration, and then subcultured into fresh growth medium (0.1 ml into 20 ml) where they were incubated with aeration for an additional 2 hours at 37° C. The bacteria were centrifuged, washed twice, and resuspended at the desired concentration in $MgCl_2$ solutions of various molarity at pH 6.8. Maximum attachment rate occurs in $5 \cdot 10^{-4}$ $M$ $MgCl_2$. Aliquots of a suspension of T1 bacteriophage in attachment medium were then added to the bacteria and the mixtures maintained at selected temperatures. Phage concentrations were determined by the standard plaque-count technique and bacterial titre by colony-count[5]. At various time intervals after mixing the phage and bacteria, 0.5 ml samples were removed from the reaction tube and centrifuged in a micro-centrifuge operating at about 5,000–10,000 $g$ for 2 minutes at the same temperature as that of the reaction tube. This procedure sediments the bacteria quantitatively, but leaves in solution all of the unattached phage, the concentration of which is then measurable by titration of an aliquot of the supernatant. In this manner the rate at which the phage attaches to bacteria can be followed. The values for the specific attachment rates and equilibrium constants as determined by this procedure are reproduceable to ± 20% in a series of experiments for which a single preparation of bacteria is used, but the reproducibility is only ± 50% when different bacterial cultures are employed, as was the case with most of the experiments here reported.

## RESULTS

*Equilibrium nature of the reversible attachment reaction.* If equation (1) correctly represents the attachment of T1 to bacteria, it should be possible to demonstrate attainment of equilibrium if the reactants are brought together under conditions where step (2) is blocked. At this point the number of T1 particles attaching to bacteria per unit time should be equal to the number dissociating from bacteria, in accordance with the equation:

$$K_{eq} = k_1/k_2 = \frac{[VC]}{[V]\ [C]} \tag{3}$$

where $K_{eq}$ is the equilibrium constant.

Fig. 1 shows the curve obtained for the reversible binding of T1 to *E. coli* strain B/1, and its irreversible interaction with *coli* B. With the resistant mutant, a steady-state is reached within 20 minutes under the conditions employed, after which the concentration of unattached T1 shows no significant change with time. If *E. coli* strain B bacteria which have been irradiated with ultra-violet light (and therefore also rendered incapable of irreversible reaction with T1)[2] are substituted for B/1 bacteria, the same attachment curve is obtained within experimental error. It can be seen from the figure that the initial rates of attachment for both cell mutants are the same.

The fact that a steady-state is reached is not sufficient, however, to prove that a state of thermodynamic equilibrium with respect to reversible attachment has been

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achieved. A critical test of this hypothesis, which is basic to the calculations that follow, is presented in Table I, where an experiment is described in which the reactants are allowed to approach equilibrium from opposite directions. The results of this experiment show that when unattached T1 is added to bacteria, the same value for the equilibrium constant is obtained as when the starting mixture contains only T1 already bound to bacteria. An additional test of the equilibrium nature of this reaction is presented in Table II, where the data show that if a mixture of T1 and bacteria which has reached

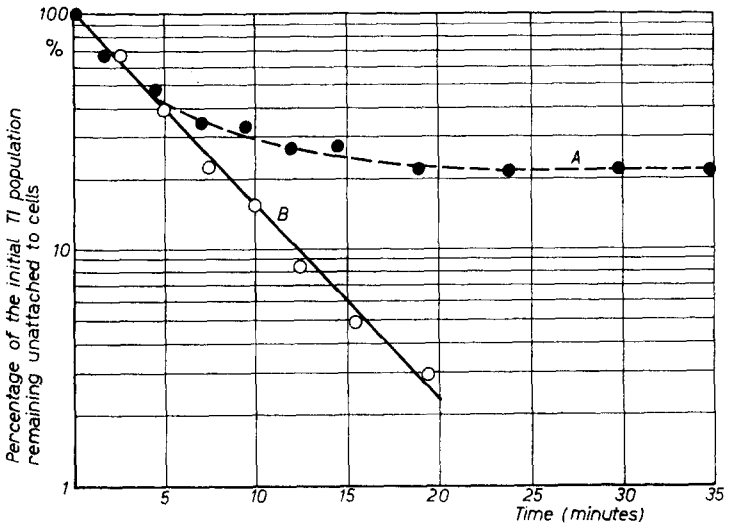


Fig. 1. The rate of attachment of T1 phage to: (A) *E. coli* strain B/1 bacteria, concentration = $2.3 \cdot 10^7$/ml; (B) *E. coli* strain B bacteria, concentration = $2.3 \cdot 10^7$/ml. Only reversible attachment occurs in (A) with *E. coli* B/1 (or with ultraviolet irradiated *E. coli* B which yields the same curve). Both reversible and irreversible attachment occur in (B) with normal *E. coli* strain B. The amount of unattached T1 was determined by centrifuging 1 ml portions of the attachment mixtures (without dilution) for 2 minutes at 37° C and titrating the supernatants for T1 phage. The rate of attachment, $k_1$, is equal to $8.0 \cdot 10^{-9}$ ml/min, as calculated from the equation $d(V)/dt = k_1(V)(C)$, where $(V)$ is the concentration of unattached T1 at a given time, and $(C)$ is the concentration of bacteria, which is a constant under the conditions of these experiments.

## TABLE I

### APPROACHING EQUILIBRIUM FROM OPPOSITE DIRECTIONS

Part A: *E. coli* strain B/1 bacteria were suspended in 2.0 ml of a medium containing $1.0 \cdot 10^{-3}$ *M* $MgCl_2$ and $2.5 \cdot 10^{-4}$ *M* sodium phosphate buffer at pH 6.8 and maintained at 37° C. A stock suspension of T1 phage was diluted by a factor of 8000 in the same medium and 0.1 ml was added to the B/1 suspension. Twenty minutes after addition of the T1, the reaction mixture was centrifuged for 3 minutes at 37° C and the supernatant was removed as completely as possible (> 95 %) and titrated for unattached T1. – Part B: The centrifuged bacteria from Part A, containing the cell-attached phage, were then resuspended in 2.1 ml of the same medium and maintained at 37° C for another 20 minute period, after which the suspension was re-centrifuged and the supernatant T1 titer again determined.

| | *Total concentration of T1* | *Concentration of E. coli B/1* | *Concentration of unattached T1 at equilibrium* | $K_{eq}$ *(bact./ml$^{-1}$)* |
|---|---|---|---|---|
| A: | $8.0 \cdot 10^4$/ml | $1.5 \cdot 10^8$/ml | $1.6 \cdot 10^4$/ml | $2.6 \cdot 10^{-8}$ |
| B: | $6.5 \cdot 10^4$/ml | $1.5 \cdot 10^8$/ml | $1.4 \cdot 10^4$/ml | $2.4 \cdot 10^{-8}$ |

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a steady-state is then diluted, the system readjusts to a new equilibrium position in accordance with the change in the concentrations of the reactants. We conclude from these experiments that the attachment of T1 to *E. coli* B/1 behaves as a thermodynamically reversible system.

TABLE II

THE SHIFT IN EQUILIBRIUM ON DILUTING THE REACTION MIXTURE

*E. coli* B/1 bacteria were suspended in a medium containing $1.0 \cdot 10^{-3}$ *M* $MgCl_2$ and $5 \cdot 10^{-4}$ *M* sodium phosphate buffer at pH 6.8. A stock suspension of T1 phage was diluted by a factor of 10,000 in the same medium and an aliquot was added to the bacterial suspension. The mixture of T1 and B/1 was kept at 37° C for 40 minutes, after which the mixture was diluted in the same medium by factors of 10 and 40 in tubes A and B, respectively. Another 40 minutes was allowed for these dilution tubes to reach equilibrium and then they were centrifuged and the supernatants titrated for unattached virus.

| *Total concentration of T1* | *Concentration of E. coli B/1* | *Concentration of unattached T1 at equilibrium* | $K_{eq}$ *(bact./ml)*$^{-1}$ |
|---|---|---|---|
| A: $4.4 \cdot 10^3$/ml | $0.72 \cdot 10^8$/ml | $1.8 \cdot 10^3$/ml | $2.0 \cdot 10^{-8}$ |
| B: $1.1 \cdot 10^3$/ml | $0.18 \cdot 10^8$/ml | $0.83 \cdot 10^3$/ml | $1.9 \cdot 10^{-8}$ |

*The degree of uniformity of the reactants.* The following experiment was run to determine the extent to which a T1 population behaves homogeneously in the reversible attachment reaction, as expressed in equation (1). A mixture of T1 and *E. coli* B/1 was allowed to reach equilibrium, and the T1 fraction which remained unattached was then isolated and added to fresh bacteria under the same conditions. The equilibrium reached with this fraction was again measured in the same manner. If the phage population contained significant numbers of particles which react more slowly, they should become concentrated, and hence detectable, in the unattached fraction. This fractionation procedure was repeated for four consecutive cycles on a given T1 stock, and the amount

TABLE III

THE DEGREE OF UNIFORMITY OF A T1 POPULATION WITH RESPECT TO THE REVERSIBLE ATTACHMENT REACTION

Cycle 1: *E. coli* B/1 bacteria were washed and resuspended in a $5 \cdot 10^{-4}$ *M* $MgSO_4$ medium at pH 7 for use as a stock bacterial suspension throughout this experiment. An aliquot of this suspension was added to the reaction tube containing a $5 \cdot 10^{-4}$ *M* $MgSO_4$ medium. At zero time an aliquot of a T1 phage stock, diluted in the same medium, was added to the reaction tube, and the mixture of T1 and B/1 was equilibrated at 37° C. After 40 minutes portions of the reaction mixture were centrifuged and the supernatants titrated for unattached T1. At 45 minutes, the remainder of the suspension was centrifuged and the supernatant removed, for use in Cycle 2. – Cycle 2: An aliquot of the bacterial stock suspension was added to the supernatant from Cycle 1 to give the same bacterial concentration as before. The reaction mixture was then treated in the same manner as above. – Cycles 3 and 4: The above procedures were repeated exactly for each succeeding cycle.

| *Cycle* | *E. coli B/1 Concentration* | *Total T1 concentration* ($10^7$/ml) | *Concentration of una tached T1 at equil.* ($10^7$/ml) | *% of original T1 remaining unattached at equil.* | $K_{eq}$ *(bact./ml)*$^{-1}$ |
|---|---|---|---|---|---|
| 1 | $7.5 \cdot 10^7$/ml | 1.1 | 0.25 | 23 | $4.6 \cdot 10^{-8}$ |
| 2 | $7.5 \cdot 10^7$/ml | 0.30 | 0.064 | 5.8 | $5.0 \cdot 10^{-8}$ |
| 3 | $7.5 \cdot 10^7$/ml | 0.067 | 0.022 | 2.0 | $2.7 \cdot 10^{-8}$ |
| 4 | $7.5 \cdot 10^7$/ml | 0.024 | 0.010 | 0.9 | $1.8 \cdot 10^{-8}$ |

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of unattached T1 at equilibrium was determined after each cycle. The results of this experiment, as listed in Table III, show that the ratio of attached to unattached T1 remains constant during the first two equilibrium cycles involving 95% of the T1 population. In the last two cycles which are listed in Table III, however, the equilibrium constant decreases. This could be caused either by a decrease in $k_1$, or an increase in $k_2$, or both, for a fraction of the phage particles. In order to determine which of these factors is responsible, 98% of a T1 stock was first reversibly attached to *E. coli* B/1, and then the residual 2% of the phage was isolated and its rate of irreversible attachment to wild type *E. coli* B was measured. As shown in Fig. 2, $k_1$ for this 2% T1 fraction is appreciably lower than that for the total population. Thus, at least part of the difference in the equilibrium behaviour of that part of the T1 with lowered cell affinity is due to a reduced efficiency of the initial, reversible attachment. The data of Table III, while demonstrating the existence of phage heterogeneity with respect to the attachment reaction, are not sufficiently sensitive to furnish a quantitative measure of the extent of such heterogeneity among the phage population[6].

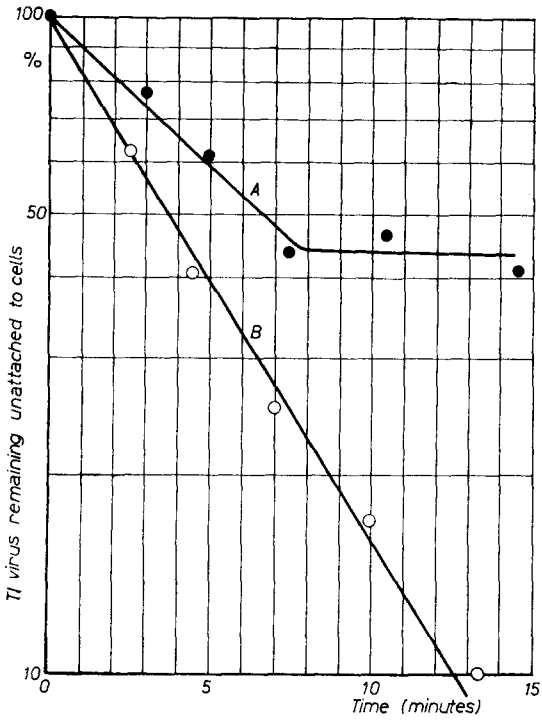


Fig. 2. Curve A: A stock of T1 bacteriophage was fractionated on *E. coli* B/1 bacteria by two equilibrium cycles as described in Table III. After the second fractionation cycle 1.5% of the original T1 population remained unattached. This fraction was then added to *E. coli* strain B cells under the following conditions: T1 concentration $6.2 \cdot 10^5$/ml; *E. coli* B concentration $4.3 \cdot 10^7$/ml; attachment medium $5 \cdot 10^{-4}$ *M* $MgSO_4$, temperature 37° C. The amount of unattached T1 was determined by centrifuging portions of the reaction mixture at the times shown (without prior dilution of the mixture) and titrating the supernatant solution for its T1 concentration. Curve B (control): The attachment rate of an aliquot of the original unfractionated T1 stock to normal *E. coli* B bacteria was determined under the same conditions used for Curve A.

A slower-attaching phage fraction was also reported twenty years ago by SCHLESSINGER[7] and later confirmed by DELBRÜCK[8]. Recent experiments of SAGIK[9] with T2 phage suggest a mechanism which may explain this inhomogeneity in the attachment reaction. He found that a variable fraction of all fresh T2 preparations attaches to cells at a very low rate because of an inhibitory material associated with the phage. After various treatments to remove this material, all of the phage particles are able to attach at the normal rate. Similar material inhibiting cell-attachment may be present in the T1 preparations.

It has not been possible to subject the bacteria to a similar test for uniformity of their virus affinity because no technique is available for separating bacteria which have bound phage reversibly from those which have not.

*Effect of varying relative concentrations of virus and bacteria.* A single bacterium is capable of binding several hundred phage particles[10]. However, in the experiments which are reported in this paper the ratio of phage to bacteria has been kept sufficiently

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low so that not more than a single phage particle attaches to any bacterium. Under these conditions the equilibrium constant, according to equation (3), should be independent of the total concentration of phage. The data in Table IV show this to be the case for a thousandfold variation in concentration of T1.

TABLE IV

CONSTANCY OF THE ATTACHMENT EQUILIBRIUM CONSTANT WITH VARYING CONCENTRATIONS OF T1

*E. coli* strain B/1 bacteria were washed and resuspended in a medium containing $5 \cdot 10^{-4}$ *M* $MgSO_4$ at pH 7 where attachment rate is maximal. A T1 stock was serially diluted in the same medium in 10-fold steps. Aliquots of the various T1 dilutions were added to tubes containing equal concentrations of bacteria, to give the concentrations of T1 as indicated. After equilibration at 25° C for 30 minutes, each tube was centrifuged at the same temperature and the supernatants were titrated for unattached T1.

| *Total concentration of T1* | *Concentration of unattached T1 at equilibrium* | *Concentration of E. coli B/1* | $K_{eq}$ *(bact./ml)*$^{-1}$ |
|---|---|---|---|
| $2.8 \cdot 10^4$/ml | $0.22 \cdot 10^4$/ml | $1.0 \cdot 10^8$/ml | $12.0 \cdot 10^{-8}$ |
| $2.8 \cdot 10^5$/ml | $0.24 \cdot 10^5$/ml | $1.0 \cdot 10^8$/ml | $11.0 \cdot 10^{-8}$ |
| $2.8 \cdot 10^6$/ml | $0.22 \cdot 10^6$/ml | $1.0 \cdot 10^8$/ml | $12.0 \cdot 10^{-8}$ |
| $2.8 \cdot 10^7$/ml | $0.24 \cdot 10^7$/ml | $1.0 \cdot 10^8$/ml | $11.0 \cdot 10^{-8}$ |

The maximum number of T1 particles capable of attachment to a host bacterium was determined by adding increasing amounts of T1 to a constant number of *E. coli* B in $5 \cdot 10^{-4}$ *M* $MgSO_4$ at 36° C, and then measuring the amount of T1 remaining unattached in the supernatant. This procedure is possible with T1, since this phage does not lyse the bacteria "from without"[8]. It was found that a maximum of 1200 T1 particle, could attach to a single bacterium. This value approximates the maximum number of phage particles which can fit in a close-packed array on the surface of the bacterium, a result to be expected on the basis of the demonstrated high collision efficiency of phage attachment reaction[7,8,1]*. WATSON[10] similarly found that T2 phage which is considerably larger in cross-section than T1, can attach only to the extent of 200–300 particles per bacterium, a value which approaches its maximum on a close-packing basis. The maximum number of phage particles capable of attaching simultaneously to a single host cell furnishes only a minimum value for the number of cellular attachment sites since a single virus could conceivably mask a large number of such sites in addition to those to which it actually binds.

Equation (3) predicts also that the equilibrium constant should be independent of the bacterial concentration when the ratio of phage to bacteria remains small with respect to the saturation value. Although the constant obtained is reasonably independent of the cell density at moderately low cell concentrations, $10^8$/ml, at higher values a definite drop is observed. This phenomenon which has been quantitatively studied by CLEVELAND[6] appears to reflect the heterogeneity of the attachment equilibrium constant of the phage population (see Table III). A drop in the rate of irreversible attachment of T4 with increasing host cell concentration has been reported by STENT AND WOLLMAN[11].

* An *E. coli* strain B bacterium in the exponential growth phase approximates a cylinder of $0.5 \cdot 10^{-4}$ cm diameter and $2 \cdot 10^{-4}$ cm length. Therefore, the available surface area is about $3 \cdot 10^{-8}$ cm$^2$. Since the head of a T1 particle is approximately a sphere of $5 \cdot 10^{-6}$ cm diameter, a close-packed arrangement of T1 on a bacterial surface could accomodate about 1500 particles.

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*Thermodynamic functions of reversible T1 binding.* Since the preceding experiments have established the reversible nature of the primary attachment of T1 to bacteria, the equilibrium for the reaction was further studied as a function of the ionic environment and the temperature. The results of these experiments are plotted in Fig. 3.

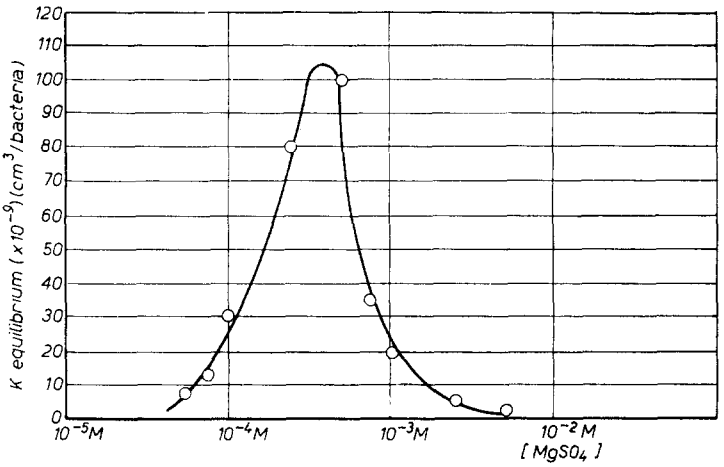


Fig. 3. The equilibrium constant as a function of ionic concentration and temperature: *E. coli* strain B/1 bacteria were washed and resuspended in solutions containing various $MgSO_4$ concentrations at pH 7, to give a concentration of $1 \cdot 10^8$/ml. A T1 stock was diluted $10^4$ times in $1 \cdot 10^{-4}$ *M* $MgSO_4$, and 0.1 ml was added to 4 ml of each bacterial tube at 37° C and the supernatant titrated for unattached T1 at equilibrium. The experiment was also run at 2° C and the resulting equilibrium constants agreed quantitatively with those at 37° C, showing that the equilibrium constant has no detectable temperature-dependence in this range.

From the data in Fig. 3, values for the standard free-energy $\Delta F^0$, the heat of reaction $\Delta H^0$, and the entropy $\Delta S^0$ may be evaluated by means of the thermodynamic relationships:

$$\Delta F^0 = -RT \ln K_{eq} \quad (4)$$

$$\Delta H^0 = (\mathrm{d}\ln K/\mathrm{d}T)RT^2 \quad (5)$$

$$\Delta S^0 = (\Delta H^0 - \Delta F^0)/T \quad (6)$$

These thermodynamic constants are listed in Table V for reversible T1 attachment in $5 \cdot 10^{-4}$ *M* $MgSO_4$, a medium permitting optimal reaction rate.

TABLE V

THERMODYNAMIC CONSTANTS FOR THE REVERSIBLE ATTACHMENT OF T1 PHAGE TO *E. coli* STRAIN B/1 BACTERIA

Medium: $5 \cdot 10^{-4}$ *M* $MgSO_4$, pH 7; T1 concentration: $1 \cdot 10^5$/ml; *E. coli* strain B/1 concentration: $1 \cdot 10^8$/ml.

| $K_{eq}$ | $\Delta F^\circ$ | $\Delta H^\circ$ | $\Delta S^\circ$ |
|---|---|---|---|
| $5 \pm 2.5 \cdot 10^{-13}$* | $-19{,}700 \pm 500$ calories/mole | $<1000$ calories/mole | $64 \pm 2$ calories/mole/degree |

* Bacterial concentration in units of moles/liter, where a mole of bacteria is defined as $6 \cdot 10^{23}$ particles.

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*Rate constants for reversible T1 binding and dissociation.* In Fig. 4 the rate of T1 attachment to normal *E. coli* strain B (which can be measured more accurately than attachment to strain B/1) is plotted as a function of ionic concentration. Since attachment rates to strains B and B/1 bacteria are comparable (as shown in Fig. 1) the values for $k_1$ in Fig. 4 have been combined with the values for the equilibrium constant from Fig. 3 to calculate $k_2$, the rate of T1 dissociation, according to equation (3). These values for $k_2$ are plotted in Fig. 4.

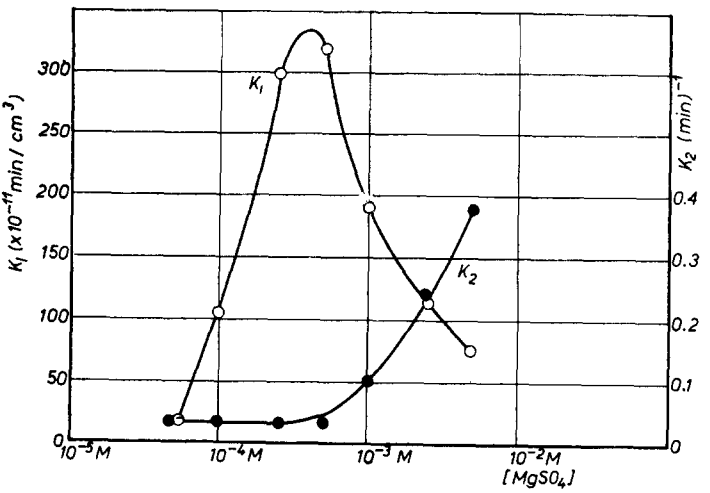


Fig. 4. $k_1$ and $k_2$ as functions of ionic concentration. Cells of *E. coli* B were washed and resuspended in solutions of various $MgSO_4$ concentrations, pH 7, to yield a bacterial concentration of $1 \cdot 10^8$/ml. A T1 stock was diluted $10^4$ times in $1 \cdot 10^{-4}$ *M* $MgSO_4$, and then 0.1 ml was added to 5 ml of each bacterial tube at 37° C. At various time intervals, an aliquot of each tube was centrifuged at 37° C and the supernatant was titrated for unattached T1. The rate of attachment, $k_1$, was calculated from the slopes of the attachment curves as described in Fig. 1. $k_2$ was evaluated by combining the above data for $k_1$ with the data for the equilibrium constant in Fig. 3, according to the equation, $K_{eq} = k_1/k_2$.

## DISCUSSIONS AND CONCLUSIONS

In these experiments we have shown that the first step in the attachment of phage T1 to host bacteria is a thermodynamically reversible reaction, yielding a standard free-energy of — 20,000 calories/mole. Since the heat of reaction is negligibly low (<1000 calories/mole) this negative free-energy is derived entirely from the positive entropy change (64 calories/mole degree). An association reaction such as phage attachment, in which a decrease in reacting units occurs, should display a negative, rather than positive, entropy change unless other compensating interactions occur. Similar positive entropy effects are observed in the binding of ions to protein molecules[12], and are interpreted as evidence that bound water is being released from the interacting surfaces. This interpretation seems plausible for the phage-bacterium reaction in view of the fact that ionic sites (carboxyl and amino)[3,4] are involved. Since such sites are strongly hydrated, water molecules should be released when attachment sites on the phage and bacterial surfaces interact.

The values for the variation of the specific rate constants of the forward and back-

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ward reactions of step (1), and of $K_{eq}$, with salt concentration plotted in Fig. 3 and 4, bring out certain aspects of the role of ions in the attachment reaction which may be summarized as follows: In the region from $0 - 5 \cdot 10^{-4}$ $M$ $MgSO_4$, the rate of increase of both $K_{eq}$ and $k_1$ is the same, and consequently $k_2$ remains constant. Thus the tendency for equilibrium to lie towards dissociation at low ionic concentrations and towards association at intermediate concentrations would appear to be a result of the effect of ionic concentration on $k_1$ alone. However, as the concentrations are raised beyond $5 \cdot 10^{-4}$ $M$, $k_2$ no longer remains constant but instead shows a continual increase. Since $k_1$ simultaneously decreases in this region, the combined effect of the opposing changes in the two rates caused the equilibrium constant to drop sharply.

These effects of ions on attachment are interpretable in terms of the electrostatic forces which exist between the charged surfaces of a phage and a bacterium. Since in the absence of ions, phage and bacteria carry a net charge of like sign[13], the resulting electrostatic barrier would prevent a sufficiently close approach of the interacting sites to permit bond formation. When the ionic concentration is raised, this barrier can be neutralized as a result of ions binding to specific surface sites or accumulating around each surface as a diffuse ionic double-layer. At a concentration of $5 \cdot 10^{-4}$ $M$ $MgSO_4$, where the rate of attachment becomes practically equal to the rate of collision between phage and bacteria, the ions in the medium must have eliminated all significant electrostatic repulsion between the reactants. Beyond this optimal concentration, the ions begin to exert another action which causes attachment to be slower and dissociation faster. We believe that this effect of higher ionic concentrations is due to a competition between the ions and the bacteriophage for the same attachment sites[1]. Equilibrium will be shifted in favour of the binding of ions to, or double layer formation about, the attachment sites, at the expense of the binding of phage, by a mass-action effect which becomes significant only at high ionic concentrations. Hence, the amount of phage becoming attached at equilibrium would then be determined by the balance of these two opposing effects of the ionic constituents of the medium.

The average amount of time which a T1 particle spends in a reversibly bound state is given by the relationship $t(\text{av.}) = 1/k_2$. When the $MgSO_4$ concentration in the attachment medium is $5 \cdot 10^{-4}$ $M$ or less, $k_2 = .05$/min, and therefore $t(\text{av.}) = 20$ minutes under these conditions. The half-life of the irreversible step (2) for T1, has been shown to be in the neighbourhood of 10–30 seconds[13b]. Hence, a 20 minute period of reversible attachment is sufficient to insure that the irreversible reaction will occur before the phage comes off the surface of the host cell. This fact explains how it is possible for the overall process of T1 phage attachment to proceed with a collision efficiency approaching 100%, although an activation energy of around 18,000 calories/mole is required for the irreversible step of the reaction. If no other factors were involved, an activation energy of this magnitude would limit the fraction of collisions between phage and bacteria leading to irreversible attachment to about 1 in $10^{13}$, and thus reduce the rate at which the bacteria become infected with phage to an insignificantly small value. It is only because a reversible attachment step, having a high collision efficiency, precedes the irreversible one and keeps the reactants together until the next step can occur, that infection can be such a highly efficient process. Since a two-step sequence of attachment to a host cell is seen to be so advantageous to T1 phage, it is not surprising to find this sequence also functioning with other bacteriophages[1,2], and in the attachment of influenza virus to red cells[14,15].

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SUMMARY

The first step in the attachment of bacteriophage T1 to *E. coli* strain B bacteria is shown to be a thermodynamically reversible reaction. From measurements of the equilibrium constant at different ionic concentrations, values for the free-energy, heat of reaction, and entropy have been calculated. Kinetic measurements on the rates of the forward and reverse parts of the attachment reaction are also reported. The significance of these equilibrium and kinetic constants for the mechanism of phage attachment is discussed.

RÉSUMÉ

La première étape de la fixation du bactériophage sur *E. coli* souche B est une réaction thermodynamiquement réversible. A partir des mesures de la constante d'équilibre pour différentes concentrations ioniques, les auteurs ont calculé les valeurs de l'énergie libre, de la chaleur de réaction et de l'entropie. Des mesures cinétiques portant sur les vitesses de réaction dans les deux sens sont également décrites. La signification des constantes cinétiques et d'équilibre pour le mécanisme de la fixation du phage est discutée.

ZUSAMMENFASSUNG

Der erste Schritt der Anheftung von Bakteriophage T1 an *E. coli*-Stamm B-Bakterien ist wie gezeigt wird eine thermodynamisch reversible Reaktion. Aus Messungen der Gleichgewichtskonstante bei verschiedenen Ionenkonzentrationen wurden die Werte für die freie Energie, die Reaktionswärme und die Entropie berechnet. Kinetische Messungen der Geschwindigkeit der Hin- und Rückreaktion der Anheftung werden ebenfalls berichtet. Die Bedeutung dieser Gleichgewichtskonstante und der kinetischen Konstante für den Mechanismus der Phagenanheftung wird besprochen.

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