Thermodynamic Treatment of Bacteriophage T4B Adsorption Kinetics

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Received for publication 17 March 1969

The activation energy was measured for the reaction of inactive phage becoming active in the presence and in the absence of L-tryptophan and was found to be about 50% lower in the former case.

It is well known that the adsorption kinetics of bacteriophage T4 follow first-order kinetics within certain limits of the bacterial concentration (1). When the bacterial concentration does not exceed 5×10^8 per ml, the rate of adsorption is $d[P]/dt = -k[B][P]$, where k is the velocity constant, $[B]$ is the bacterial concentration, and $[P]$ is the bacteriophage concentration. If the bacterial concentration is higher than 5×10^8 per ml, the rate of adsorption does not increase as a function of the number of collisions but rather remains constant (1, 11). To explain deviations from simple collision theory, four models have been proposed.

Sequential model. Puck, Garen, and Cline (10) suggested that the first step in phage adsorption is a reversible one and that at high bacterial concentrations the second step, from reversible to irreversible adsorption, becomes rate-limiting: phage + bacterium $\rightleftharpoons$ reversible complex $\rightarrow$ infected bacterium.

Competitive model. Stent and Wollman (11) suggested an alternative model in which a collision can be "good" and irreversible or "bad" and reversible. At high bacterial concentrations, the number of "bad" collisions would therefore be rate-limiting: reversible complex $\rightleftharpoons$ phage + bacterium $\rightarrow$ infected bacterium.

Modified sequential theory. Christensen (4) found that the sequential model and the competitive model lead to certain kinetic predictions. From his experiments on bacteriophage T1, he concluded that neither model is correct but that the experimental results can be correlated with a combination of the two models: reversible complex (b) $\rightleftharpoons$ free phage $\rightleftharpoons$ reversible complex (a) $\rightarrow$ infected bacterium.

Active-inactive model. This model was also proposed by Stent and Wollman (11), and it is the only model that does not rely on the occur-

rence of a reversible complex. They suggest that phages can exist in two states, active and inactive, and that they oscillate between these two states. At high bacterial concentrations, it is the frequency of this oscillation that becomes rate-limiting.

Since it has been shown that T4 has tail fibers in either "active" or "inactive" configuration depending on the environment, I favor the last model. Therefore, the system studied was: inactive phage $\rightleftharpoons$ active phage $\rightarrow$ infected bacterium.

If the active-inactive model is the correct one, then at any one instant there should be a fraction of the population that is active and a remaining one that is not. The active form would adsorb to bacteria at a rate that is proportional to the number of collisions with bacteria, but the disappearance of the inactive form would depend on the transition rate from the inactive to the active form. If this is the case, in principle it should be possible to maximize the difference between the two groups by increasing the bacterial concentration and lowering the temperature. The high bacterial concentration would increase the rate of adsorption of the active fraction, whereas the low temperature would decrease the rate of transition from the inactive form to the active one.

Escherichia coli B was grown in glycerol-Casamino Acids medium (7) to a titer of 5×10^8 per ml, concentrated 10-fold, and allowed to equilibrate to a desired temperature for 10 min with 60 μ g of L-tryptophan per ml.

T4Bo1, a tryptophan-requiring phage, was allowed to equilibrate to temperature for 45 min in glycerol-Casamino Acids medium with 60 μ g of L-tryptophan per ml. After addition of phage, with a multiplicity of infection of 0.01, samples were taken at intervals and diluted in saline (to inactivate the unadsorbed phage); the infective centers were plated on minimal plates. The number of infective centers was

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directly related to the number of adsorbed phage. Infective centers formed plaques on indicator bacteria without the addition of L-tryptophan, indicating that newly formed phage are active (3). Figure 1 shows the results of such an experiment. It is clear that two slopes exist for each temperature, the earlier one always being greater than the later one. The striking result is that the phage population acts as if it were heterogeneous; similar experiments at higher temperatures and lower bacterial concentration do not show this heterogeneity of adsorption. This heterogeneity demonstrates that the population consists of two major fractions and that one fraction is more active than the other. Stent and Wollman (11) calculated the change in diffusion constant over the range of temperatures used in this study and found that it could not account for the changes in adsorption rate.

From the fact that the majority of the phages, for example, 95% at 8°C, are in the inactive form, even in the presence of tryptophan, it is concluded that the equilibrium is towards the inactive form.

Important information about a mechanism of a reaction can be obtained from the study of reaction rates as a function of temperature. Simple chemical reactions have been shown to follow the Arrhenius equation $d \ln k / dT = A/RT^2$ where A is the activation energy, k is the rate constant, T is the absolute temperature, and R is the ideal gas constant.

FIG. 1. Adsorption of T4Bo1 in the presence of tryptophan. Rate for formation of T4Bo1 infective centers as a function of temperature. T4Bo1 was added at a multiplicity of 0.01 to *E. coli* B at a concentration of $5 \times 10^6 \text{ ml}^{-1}$ in glycerol-tryptophan medium.

Integration yields an equation of the form $\log K/1/T = -A/2.303R + \text{constant}$. Therefore, if $\log k$ is plotted as a function of $1/T$, the slope is equal to $-A/2.303R$.

An Arrhenius plot, determined by using the rate-limiting rate constant k obtained from Fig. 1, is given in Fig. 2. [It has been shown by Stent and Wollman (11) that, at bacterial concentrations higher than 5×10^8 per ml, the rate of adsorption is independent of the bacterial concentration. Therefore, the rate of adsorption is only a function of the phage concentration; i.e., it follows first-order kinetics. The rate constants here were calculated in the conventional way derived for first-order kinetics.] The curve is linear, and from its slope a free energy of activation of 17.6 kcal is calculated. The fact that the curve is linear indicates that the transition from inactive to active is mediated through a single, temperature-sensitive step.

Unfortunately, there does not exist a straightforward method for calculating activation energy in the absence of tryptophan, since the fraction of active phage is so small compared to the extensive background and short-time adsorption kinetics cannot be used. Therefore, an indirect method, namely, counting the increase in the number of T4Bo1 plaques on plates as a function of incubation temperature, was used.

T4Bo1 was added to indicator bacteria on minimal plates in the absence of tryptophan, and these plates were incubated at different temperatures. As found by Anderson (2), the number of plaques was larger on plates incubated at high

FIG. 2. Activity of T4Bo1 in the presence of tryptophan. An Arrhenius plot determined by using the rate-limiting rate constant k obtained from Fig. 1.

temperatures. It has been assumed that the fraction of active to inactive phage is proportional to the rate constant going from inactive to active at any one temperature. This fraction of active to inactive phage was determined from the number of plaques appearing on minimal plates as compared to the number of plaques appearing on tryptophan plates. The fractions were normalized (which, of course, does not change the value of the slope), and Fig. 3 shows an Arrhenius plot of this data. In the experiments leading to the adsorption kinetics shown in Fig. 1, we used liquid Casamino Acids medium, whereas the plating experiments here were done on M9 minimal plates (1). Independent experiments have shown, however, that the rate of adsorption is the same in both M9 minimal and Casamino Acids medium, which allows a comparison of the data represented in Fig. 2 with the results given in Fig. 3.

Again, there is a linear relationship, indicating

FIG. 3. Activity of T4Bo1 in the absence of tryptophan. An Arrhenius plot determined by using the absolute increase in active phage as a function of temperature, as determined by plate assays. From the slope of the linear curve, the free energy of activation is calculated. The normalized fraction of active to inactive at different temperatures is represented by k .

a single, temperature-sensitive step in the transition from the inactive to the active. The slope yields an activation energy of 29.6 kcal or an increase of almost two.

The evidence presented suggests that the effect of L-tryptophan is one of lowering the activation energy between the active and inactive forms. It seems that the fraction of inactive to active form is large in either the presence or absence of L-tryptophan, but, in the presence of both bacteria and L-tryptophan, the active phage are removed continuously from the population. It is clear from the work of Kellenberger et al. (9), Cummings (5), and Gamow and Kozloff (8) that the inactive form consists of phage that have their fibers close to the body of the phage. Active phage may consist of individuals that have their fibers free and thus can be found in a number of random positions.

I thank L. Kozloff for many helpful discussions concerning this work.

This investigation was supported by Public Health Service grants AI 06336 from the National Institute of Allergy and Infectious Diseases and 5TO1 GM01379 from the National Institute of General Medical Sciences.

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