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ABOUT THE ADSORPTION MECHANISM OF BACTERIOPHAGE T1

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SUMMARY

The author estimates 10,000 cal/mol as the characteristic temperature of T1Hr bacteriophage adsorption by heat inactivated *E. coli*. The results obtained here, compared with those of authors working with T1 phage adsorption by viable *E. coli* cells lead the A. to suggest the participation of a thermolabile substance. Such a substance would probably be of an enzymatic nature and would be found both in the bacteriophage T1Hr tail tips and at the specific receptor leve for T1 on the *E. coli* surface.

INTRODUCTION

In a previous paper (SALLES [5]) we studied the behavior of the T1 phage and of its Hr mutant, with reference to their capacity for irreversible adsoption in *E. coli* bacteria inactivated by heat. Whe showed there that T1Hr is adsorbed irreversibly in heat inactivated bateria, but that $T1Hr^+$, which we will cal T1, is not.We also showed that T1 adsorption is derectly proportional to the number of viable bacteria in the reagent mixture. "Viable" is here defined as the bacteria's capacity to form colonies when seeded in and adequate medium. We mean by "adsorption" that phase of viral infection which lasts from the phage's movement toward the bacteria until the beginning of the DNA viral injection. Adsorption is divided in two phases: the first is essentially reversible (phase I) and the second is irreversible (phase II). Thus, for us adsorption is slightly different from the process as viewed by the physicist.

These results conform to the views of several authors, notably WEIDEL [6], according to whom cellular viability is a necessary condition for irreversible adsorption (phase II) of T1. A possible mechanism of this dependence would be the necessary participation of bacterial enzymes in one of the final steps of adsorption.

Our previous paper showed, however, that in one mutant the participation of the bacterial metabolism was not required. In this case adsorption took place as though T1Hr contained all the enzymatic equipment necessary. In order to verify this hypothesis we carried out the work presented here, in which we estimate the activation energy (Ea) involved in the T1Hr + heat inactivated bacteria reaction.

MATERIAL AND METHODS

1. Bacteriophage T1Hr, mutant "host range" of T1, from the T1-T7 series; original stock of C. Bresch. Lysates were obtained and preserved in simple broths.

2. Bacteria *E. coli* B. from the same stock. For obtention of standard culture, lysates, formula of media used, preparation of heat inactivated bacteria, titration of phages and bacteria, see SALLES [7].

3. Calculation of Ea according to the Arrhenius formula:

$$\ln K2/K1 = Ea\ (T2\text{-}T1) / R.\ T1\ .\ T2$$

where K1 and K2 are the adsorption rates of the phage at temperature T1 and T2 res-

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pectively (in Kelvin degrees), R is the constant of perfect gases equal to 1,987 cal and Ea is the activation energy.

This formula in a linear presentation permits an adjustment of the curve by means of the minimum square method. The adjustment is necessary particularly because at about 30-37°C the curve deviates. Our calculation of Q10 at above 30°C was obtained in this manner.

4. Calculation of K (constant of phage adsorption). This indicates the speed with which the phage attaches to the bacteria. It is obtained according to the following formula:

$$N/N_0 = e^{-Kt}$$

N is the free phage title unadsorbed after time t . N_0 is the phage title when time $t = 0$, and e is the base of natural logarithms.

For the technique of adsorption and disadsorption see the previous paper. The only alteration was that temperature needed for estimating diverse K (from 15-37°C) and for the suspensions of phage and bacteria were maintained in the double boiler for a long time before contact, in order to guarantee the homogeneous temperature of the reaction. The temperature varied during the experiment more or less 1°C.

RESULTS

In table I we see the adsorption rate at different temperatures. The measurement of K at temperatures below 10°C was influenced by the imprecision of the method (by free virus) used to estimate adsorption, and at above 80°C there is great fluctiation.

TABLE I

Temperature °C	K
37	0.26
35	0.24
30	0.23
25	0.20
20	0.17
15	0.14

With the data from table 1 we constructed the curve shown in fig. 1. Here one can see that the Arrhenius formula expresses perfectly the activation energy of T1Hr in heat inactivated bacteria for a considerable interval. However, at about 30°C there is a deviation frequently found by authors working with various types of biological material: seeds, anaerobious spores, etc. (MEHL & WYNNE [3]). CROZIER suggest the existence of cathenary reactions each of which has a maximum speed limit and this limit acts as a brake on the total reaction. In our case the limit would be given by the maximum rate of reversible adsorption (phase I) which is not sensitive to temperature variation (GAREN & PUCK [1]) and is responsible for the inflection of the curve in fig. 1 at about 30°C.

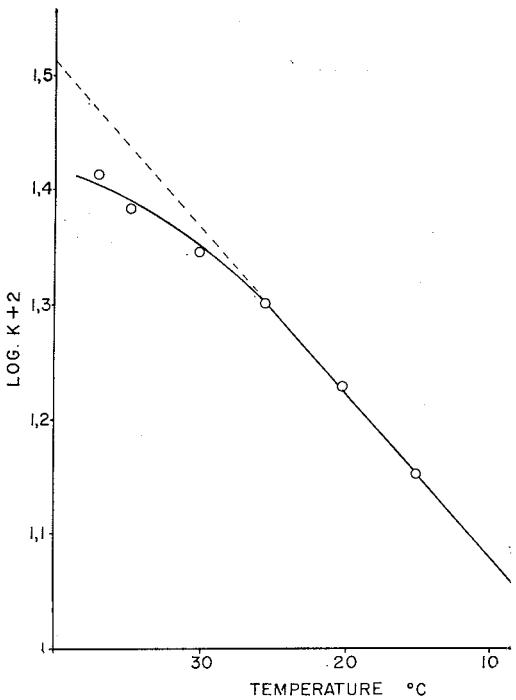

Fig. 1 — The adsorption rate (K) of T1Hr bacteriophage in the T1Hr + heat inactivated bacteria reaction, plotted against temperature. The dotted line is the corrected curve which serves for the calculation of Q_{10}. Note the deviation at about 30°C due to phase II of adsorption which is not sensitive to temperature variation.

In table II we see some Q_{10} calculated from the corrected values of K. With these experimental data we obtained an activation energy of the order of 10,000 cal/mol.

TABLE II

ΔT	Q_{10}
35 — 25	1.75
30 — 20	1.63
25 — 15	1.57

Our result is of the same order as that obtained in the adsorption of T1 by viable bacteria, where an Ea of 18,000 cal/mol was observed (PUCK *et al.* [4]). These figures suggest an enzyme type of reaction. In the case of the T1 + viable bacteria reaction, these results were expected since it was thought that live bacteria furnished the enzymes necessary for the phage attachment. Moreover, at the time these first calculations were made, little was known of the enzymes in the bacteriophage's tail tips and so, if an enzyme was involved in the adsorption reaction, it was attributed to the bacteria. At present, particularly after Kosloff's work, it is known that the phage's tail has complex enzymatic equipment and we are not surprised to find this type of reaction between an active virus and a dead bacteria.

The participation of enzymes in the T1Hr + heat inactivated bacteria reaction means that we must re-examine the adsorption mechanism of this phage. In this case the bacteria was inactivated and its enzymatic equipment denatured. Thus, any enzyme participating in the reaction would necessarily come from the phage. On the other hand, this particular phage is the "host range" mutant of T1 and is capable of infecting E. coli B/1 bacteria resistant to T1. A characteristic common to both B/1 and heat inactivated bacteria might be significant; both are attacked by T1Hr and immune to T1. The reaction proceeds as though the latter needed an X substance which normally existed in the bacteria B receptors. By mutation to B/1 * of after inactivation (by heat, high doses of UV, specific inhibitors like Zn^{++}, etc.), this substance X would be destroyed or no longer synthesized by the bacteria. Resistance to phage T1 would be a "visible" consequence of this mechanism. T1Hr, on the contrary, already possesses the substance X and due to this can be irreversible adsorbed (phase II) by denatured *E. coli* bacteria or B/1 mutants.

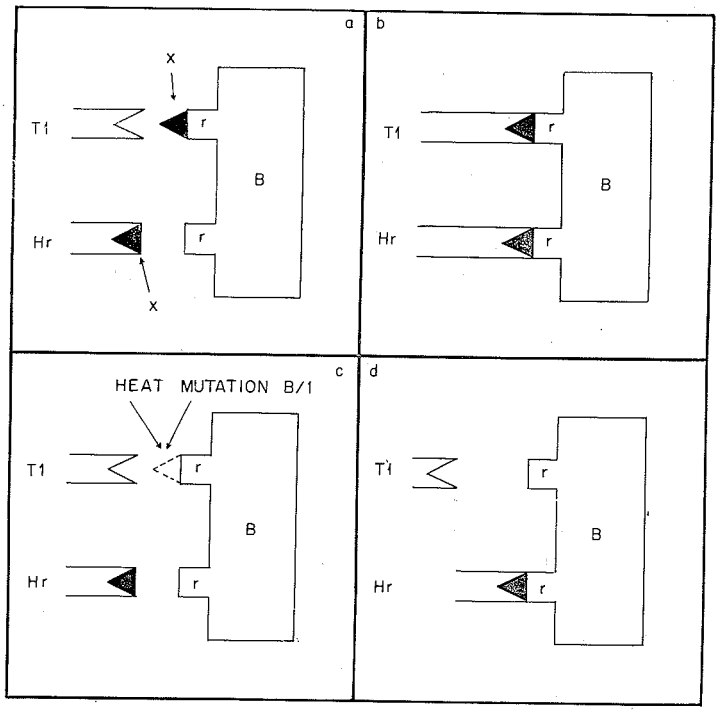

Fig. 2 — In *a* we see tail tips of two phages, T1 and Hr, about to adsorbed by receptors (r) of bacteria (B). In *b* they are irreversibly adsorbed. In *c* we have the same situation as in *a* but the specific receptor for T1 of bacteria (B) is lacking substance X which has been destroyed by heat or which is absent by mutation to B/1. In *d* the situation represented in *b* is repeated but T1 has not been adsorbed due to the lack of substance X.

A schematic view of this hypothesis is presented in fig. 2.

It is not probable that this substance is assimilated to the receptors since these are extraordinarily resistant to denaturtion, as the work of WEIDEL & KELLENBERGER [7]

* But not B/1.5 (see GAREN & PUCK [1]).

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shows. It remains to examine the hypothesis stated in the discussion of the previous work. We adopted there a dual point of view. We suggested a double mechanism for the adsorption of T1Hr: one operating for bacteria B and B/1 and another for heat inactivated bacteria. In view of the result of work presented here, in which the Ea of the T1Hr + heat inactivated bacteria reaction is equivalent to the Ea of the T1 + viable bacteria reaction, a double mechanism appears to operate both for bacteria and bacteriophage. The bacteria undergoes two types of reaction: type B and type heat inactivated bacteria (or B/1). The phages present two types of mechanism: type T1 and type T1Hr.

One direct consequence of this double mechanism is that the efficiency of plating might vary both by phage and by bacteria mutation, not only in an "all or nothing" manner but also gradually.

If X were a mixture of substances of different types of activity, or even if it were homogeneous but of variable activity according to the number of molecules available for each phage or receptor, there is a possibility that mutants of an entire range of efficiency of plating might be found in both bacteria and bacteriophage. This would occur in cases where the X substance is necessary to adsorption (phase II) as the case of T1 and perhaps T7 (MACKAL & KOZLOFF [2]).

The recent work of HAUSSMAN *et al.* (personal communication) with T7 mutants characterized by different efficiency of plating indicates this.

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RESUMO

Sôbre o mecanismo de adsorção do bacteriófago T1

A energia de ativação, para a adsorção do bacteriófago T1Hr por *Escherichia coli* inativada pelo calor, foi estimada em 10.000 cal/mol. Os resultados aqui obtidos, quando comparados com os de outros autores relativos à adsorção do fago T1 pela *E. coli* viável, levaram o A. a sugerir a participação de uma substância termolábil no processo. Esta seria provàvelmente de natureza enzímica e seria encontrada tanto na ponta da cauda do bacteriófago T1Hr ocmo ao nível do receptor específico para T1, na superfície de *E. coli.*

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